The Journal of Parasitology

Volume 40 JUNE, 1954 Number 3

SEXUAL ISOLATION BETWEEN POPULATIONS OF THE *CULEX PIPIENS* COMPLEX IN NORTH AMERICA*

LLOYD E. ROZEBOOM AND BARBARA N. GILFORD

In recent years, a small group of workers scattered throughout the world has become interested in the population genetics and other aspects of the biology of the mosquitoes belonging to the *Culex pipiens* complex. A resumé of the findings and opinions of some of these investigators was given in a symposium held at a meeting of the Royal Entomological Society of London (see Mattingly *et al.,* 1951). In North America there are at least three recognizable populations: *C. pipiens* in the north; *C. quinquefasciatus* in the south; and *C. molestus,* which coexist in several localities at least with *pipiens.* A possible fourth population is *dipseticus* in the southwest. Work in this laboratory has led us to believe that *pipiens* and *quinquefasciatus* should be regarded as subspecies (Farid, 1949; Sundararaman, 1949; Barr and Kartman, 1951). We prefer for the present to think of *molestus* as being a "biotype" in the sense that to us it appears to represent a group of variant individuals, which in the laboratory or under special conditions in nature may breed true as long as mechanisms for the selection of autogenous individuals remain in operation.

We have not been able to find any genetic or other barriers between *C. pipiens, quinquefasciatus,* and *molestus* populations; yet they seem to maintain themselves as fairly distinct entities in nature. There is a small but recognizable morphologic difference between the male terminalia of *pipiens* and *quinquefasciatus,* and Knight (1953) has shown that in general the larvae of these two forms may be separated by the branching of hair no. 4 of abdominal segment III. *C. molestus* is unique in its ability to lay eggs without a blood meal. In the apparent absence of more obvious isolating mechanisms, the question arises as to whether gene-flow between the three kinds of mosquitoes might be blocked or at least materially reduced through mating barriers.

The purpose of this study was to determine whether sexual isolation might exist between populations of *C. pipiens, quinquefasciatus,* and *molestus* in North America.

MATERIALS AND METHODS

The mosquitoes were obtained from colonies maintained in our insectary. The forms used were as follows:

Culex pipiens, originating from material collected in the vicinity of Baltimore.

Received for publication, September 21, 1953.

*From the Division of Medical Entomology, School of Hygiene and Public Health, the Johns Hopkins University. This study was supported in part by a research grant from the Division of Research Grants, National Institutes of Health, Department of Health, Education, and Welfare.

Culex quinquefasciatus, two colonies, one originating from material collected at Galveston, Texas, and the other in Montgomery, Alabama.

Culex molestus, from Baltimore, established by Dr. R. Wallis from a single female. Work in progress by Mr. A. Spielman has shown that this colony is essentially 100 per cent autogenous.

All experiments were carried out in an air-conditioned insectary, with the temperature maintained at approximately 80° F. The pupae taken from the colonies were isolated in test tubes and the adults were allowed to emerge in these tubes, so that there was no chance of mating before the experiments were set up. After emergence, the adults were placed in lantern chimneys (males and females in separate chimneys) for further aging before they were used. These adults were one to three days old before they were placed in the breeding cages, and the sexes were left together in the cages for periods of two to four days. Contact between the sexes should thus have been maintained during what may be presumed to be an especially active period in the adult mosquitoes' lives. During this period of contact, the adults were fed on sugar water, and humidity was kept at a high level by placing a cylinder of wet towels, inserted in a beaker containing water, inside the cage, and a layer of wet towels over the top.

In most of the experiments, the breeding cages had a capacity of one cubic foot. In a few experiments, cages 23 by 22 by 19 inches in size were used.

Two types of experiments were conducted. In one, males had an opportunity to select between two kinds of females; in the other, males were placed in contact with only one kind of female. The mating selection experiments were conducted in the following manner. Ten males, for example, of *C. pipiens,* and 25 females each of *pipiens* and *quinquefasciatus* were placed in a breeding cage, and at the same time 10 male *quinquefasciatus* were placed in another cage with 25 each of *quinquefasciatus* and *pipiens* females. This procedure was repeated with all combinations of the four colonies. The 1 : 5 male to female ratio was employed in order to prevent the obscuring of any possible selection by too great male activity. Following the two to four day period of contact in the cages, the mosquitoes were removed and the females dissected and examined microscopically for the presence of spermatozoa. The criterion for successful mating was considered to be the presence of spermatozoa in the spermathecae.

Prior to being placed in the breeding cages, the females were marked with bronze or aluminum powder; this insured accurate identification at the time of dissection. The dusting was light enough to prevent any interference with the mosquitoes' activities; also, the dusts were alternated in the replicates of the experiments.

In the non-selection experiments, the males were placed in breeding cages with females of only one kind. This type of experiment was set up as follows: 10 males of *C. pipiens* were placed in each of three breeding cages; 50 females of *pipiens* were placed in one cage; 50 *quinquefasciatus* females were placed in another, and 50 *molestus* females in the third cage. This was repeated for each kind of male, and each experiment was repeated once. In most of these tests, it was not possible to use both strains of *quinquefasciatus;* however, in two of the replicates, sufficient material of both strains was available, and in these cases, four breeding cages were set up simultaneously.

The results of the selection experiments are presented in tables 1–6. The differences in the percentages of females inseminated are preceded by a plus sign when the homologous matings are larger, and by a minus sign when they are smaller. Differences in proportions were tested for significance by use of the formula $\sigma_D\% = \sqrt{pq\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$; in the tables, all differences are highly significant (for the most part, $p = < .001$), except those marked with an asterisk.

EXPERIMENTAL OBSERVATIONS

Experiment 1. In this experiment an opportunity for selection was provided between sexes of *C. pipiens* and the Galveston strain of *C. quinquefasciatus.* The experiment was performed four times; in each replicate the 1-cu. ft. breeding cages were utilized. The results are summarized in table 1. The *pipiens* males and females exhibited a rather low degree of sexual activity, and the difference between the numbers of *pipiens* and *quinquefasciatus* females inseminated by the *pipiens* males was not great enough to be considered significant. Furthermore, this difference was due to the results obtained in two of the replicates; in the other two tests a few more *quinquefasciatus* than *pipiens* females were inseminated by the *pipiens* males. On the contrary, a significantly higher proportion of *quinquefasciatus* females were inseminated by the *quinquefasciatus* males; in this case there were no reversals in the results of the replicates.

TABLE 1.—C. pipiens × C. quinquefasciatus (*Gal.*)

Males	Females				Diff.
	pipiens		quinque		
	No.	% pos.	No.	% pos.	
pipiens	91	20.9	90	11.1	+9.8*
quinque	97	8.3	93	63.4	+55.1
Diff.		+12.6		+52.3	

Summary of 4 replicates.
* p = .07.

Experiment 2. In this experiment, the degree of selective mating was determined between *C. pipiens* and the Montgomery strain of *C. quinquefasciatus.* This combination was tested five times. The results of all replicates were consistent; they are summarized in table 2. One may note that three times as many *quinquefasciatus* as *pipiens* females were inseminated by the *pipiens* males; that more *pipiens* females were inseminated by *quinquefasciatus* than by *pipiens* males; and that three times as many *quinquefasciatus* as *pipiens* females were inseminated by the *quinquefasciatus* males.

TABLE 2.—C. pipiens × C. quinquefasciatus (*Mont.*)

Males	Females				Diff.
	pipiens		quinque		
	No.	% pos.	No.	% pos.	
pipiens	120	12.5	119	37.0	−24.5
quinque	112	19.6	118	65.3	+45.7
Diff.		−7.1*		+28.3	

Summary of 5 replicates.
* p = .13.

TABLE 3.—C. pipiens × C. molestus

Males	Females				Diff.
	pipiens		molestus		
	No.	% pos.	No.	% pos.	
pipiens	91	18.7	94	72.3	−53.6
molestus	95	29.5	91	92.3	+62.8
Diff.		−10.8*		+20.0	

Summary of 4 replicates.
* p = .09.

Experiment 3. Four mating selection tests were run with the *pipiens* and *molestus* combination. One of the tests was run with the large breeding cages, and the other three in the 1-cu. ft. cages. There was no difference in the degree of sexual activity in the large and small cages. There were no reversals in the results obtained in the replicates; these results are summarized in table 3, and they show that *pipiens* males as well as *molestus* males inseminated approximately three times more *molestus* than they did *pipiens* females. More *pipiens* females were inseminated by *molestus* males than by their own males.

Experiment 4. The *C. molestus* and the Galveston strain of *C. quinquefasciatus* combination was tested four times. In all of these replicates the 1-cu. ft. breeding

TABLE 4.—C. molestus × C. quinquefasciatus (*Gal.*)

Males	Females				Diff.
	molestus		quinque		
	No.	% pos.	No.	% pos.	
molestus	98	93.9	90	16.7	+77.2
quinque	88	64.8	81	21.0	−43.8
Diff.		+29.1		+4.3*	

Summary of 4 replicates.

cages were employed. Results obtained are summarized in table 4. This table shows that significantly higher percentages of *molestus* females were inseminated by both the *molestus* and *quinquefasciatus* males. More *molestus* females were inseminated by their own than by *quinquefasciatus* males, but there was no significant difference between the numbers of the two kinds of females inseminated by the *quinquefasciatus* males.

Experiment 5. In this experiment the Montgomery strain of *C. quinquefasciatus* was tested with *C. molestus.* There were five replicates, of which one was run in the large cages, and the other four in the 1-cu. ft. cages. The size of the cages had no effect on the results, and the results of the replicates were very similar. They are summarized in table 5. A significantly higher proportion of *molestus* females was inseminated by both *molestus* and *quinquefasciatus* males, and essen-

TABLE 5.—C. molestus × C. quinquefasciatus (*Mont.*)

Males	Females				Diff.
	molestus		quinque		
	No.	% pos.	No.	% pos.	
molestus	100	95.0	104	35.6	+59.4
quinque	115	71.3	107	32.7	−38.6
Diff.		+23.7		−2.9*	

Summary of 5 replicates.

TABLE 6.—C. quinquefasciatus (*Mont.*) × C. quinquefasciatus (*Gal.*)

Males	Females				Diff.
	Mont.		Gal.		
	No.	% pos.	No.	% pos.	
Mont.	94	34.0	97	15.5	+18.5
Gal.	83	39.9	96	18.8	−21.1
Diff.		−5.9*		+3.3*	

Summary of 4 replicates.

tially the same proportion of the *quinquefasciatus* females was inseminated by the two kinds of males.

Experiment 6. Because the Montgomery and Alabama strains of *C. quinquefasciatus* exhibited different degrees of sexual activity in the previous trials, the purpose of this experiment was to make additional tests for differential activity and for selectivity between these strains. There were four replicates of this experiment, and all of them were run in the 1-cu. ft. breeding cages. The results of the replicates were all similar; they are summarized in table 6. This table shows that approximately twice as many females of the Montgomery strain were inseminated by both strains of males. The degree of sexual activity exhibited by any one of the two strains of females was the same regardless of the strain of males present.

Experiment 7. The purpose of this experiment was to determine whether the rate of insemination would be affected by the presence of females of only one kind. For example, in Experiment 1, *pipiens* males inseminated twice as many *pipiens* as they did *quinquefasciatus* females in cages containing both kinds of females, and it would be of interest to determine whether more of the *quinquefasciatus* females would have been inseminated had no *pipiens* females been available to the males.

The results of this experiment are given in table 7. As it is desirable to refer to the results of the individual tests, this table has not been condensed. A comparison of the selection and non-selection experiments is facilitated by setting the results of the two types of experiment against one another in a single table. This has been done in table 8. It will be noted that the percentages of females inseminated by their own males were approximately the same regardless of the presence or absence of females of another kind. In some of the replicates there seemed to be a real difference; thus, 64.6% of the Montgomery *quinquefasciatus* females were inseminated by the Galveston *quinquefasciatus* males in the non-selection experi-

TABLE 7.—*Per cent insemination of females in non-selection experiments*

Males	Exp. No.	pipiens		quinque (M.)		quinque (G.)		molestus	
		No.	% pos.	No.	% pos.	No.	% pos.	No.	% pos.
pipiens	7a	44	13.6	50	32.0			50	36.0
	7b	44	20.4	48	20.8			50	72.0
	Total	88	17.1	98	26.5			100	54.0
quinque (Mont.)	7c	47	32.0	49	55.0			49	61.0
	7d	49	28.6	49	57.2			48	56.2
	Total	96	30.2	98	56.1			97	58.8
quinque (Gal.)	7e	46	21.8			36	52.8	44	70.5
	7f	47	8.5	48	64.6	50	62.0	49	69.4
	Total	93	15.1	48	64.6	86	58.2	93	69.9
molestus	7g	48	31.3	48	29.2	41	26.8	47	89.4
	7h	50	26.0			39	38.5	44	86.4
	Total	98	28.6	48	29.2	80	32.5	91	87.9

ment, while only 39.9% of these females were inseminated by the Galveston males in the presence of the Galveston females. In the *pipiens* × *quinquefasciatus* combination (column 1 of table 8), more *pipiens* females were inseminated by both strains of *quinquefasciatus* males when they were the only females available to the males. On the other hand, 37% of the Montgomery *quinquefasciatus* females were inseminated by the *pipiens* males in the presence of the *pipiens* females, but only 26.5% when the *pipiens* females were absent. Similar reversals will be found in the results obtained with other combinations.

TABLE 8.—*Comparison of selection and non-selection experiments*

Males	Per cent insemination of females							
	pipiens		quinque (G.)		quinque (M.)		molestus	
	Sel.	Non-sel.	Sel.	Non-sel.	Sel.	Non-sel.	Sel.	Non-sel.
pipiens	20.9 12.5 18.7	17.1	11.1	—	37.0	26.5	72.3	54.0
quinque (Gal.)	8.3	15.1	63.4 21.0 18.8	58.2	39.9	64.6	64.8	69.4
quinque (Mont.)	19.6	30.2	15.5	—	65.3 32.7 34.0	56.1	71.3	58.8
molestus	29.5	28.6	16.7	32.5	35.6	29.2	92.3 93.9 95.0	87.9

DISCUSSION

A number of studies have been made on sexual isolation between species and races of *Drosophila* (see Dobzhansky, 1951), but we seem to have little information concerning the existence of this type of barrier between mosquito populations, in spite of the fact that a number of species of public health importance are members of species complexes. Bates (1949) has presented evidence that a certain amount of selective mating takes place between *Anopheles atroparvus* males and females in the presence of *maculipennis* females, but not in the presence of *labranchiae* females. Certainly, the differences in mating behavior of the species and subspecies of the *A. maculipennis* complex, which have been studied by a number of investigators, should function as one of the mechanisms whereby these populations remain separated from one another.

Among the North American *Anopheles,* degrees of sexual isolation exist between *A. quadrimaculatus, freeborni,* and *aztecus* (Rozeboom, 1953). These, however, are distinct though closely related species, and the mating barriers may have developed along with genetic ones after long periods of geographic isolation. In the case of the *pipiens* complex we are dealing with forms which we believe have not attained specific status and whose ranges overlap in many areas.

The results of a preliminary experiment with *Culex pipiens* and *C. quinquefasciatus* indicated that these mosquitoes were totally unable to recognize the opposite sex of their own kind (Rozeboom, 1951, in Mattingly, *et al.*). The more extensive experiments of the present study might suggest that this conclusion was incorrect. To illustrate, the fact that a significantly higher proportion of *quinquefasciatus* fe-

males in Experiment 2 was inseminated by their own males would indicate that the *quinquefasciatus* males were discriminating against the *pipiens* females. However, more *quinquefasciatus* than *pipiens* females were inseminated by *pipiens* males. This could be interpreted as a discrimination on the part of the *pipiens* males against their own females. Thus it is evident that the differences in insemination rates were due not to selection, but to a greater sexual activity exhibited by the *quinquefasciatus* males and females.

Tables 3–6 all show similar reversals.

Table 8 shows that the rate of insemination does not seem to be particularly affected by the presence of females belonging to another population. The results of the non-selection experiment (table 7) do not reveal an ability of the sexes of a given population to recognize one another. It is true that more *quinquefasciatus* females were inseminated by the *quinquefasciatus* males than were *pipiens* females; however, more *quinquefasciatus* than *pipiens* females were inseminated by the *pipiens* males. More *molestus* females were inseminated by *pipiens* and *quinquefasciatus* males than were *pipiens* or *quinquefasciatus* females.

Table 7 does show a difference in sexual activity between the populations. If one compares the insemination rates of each kind of female in the vertical columns of table 7, it will be observed that from 8.5 to 32% of the *pipiens* females, 20.8 to 64.6% of the Montgomery *quinquefasciatus* females, 26.8 to 62% of the Galveston *quinquefasciatus* females, and 36 to 89.4% of the *molestus* females, were positive. These differences might be made more clear by presenting the means and standard deviations of the positive percentages of the vertical columns, but these would be biased because it was not possible to include the Galveston *quinquefasciatus* in the tests with the less active *pipiens* males.

Dobzhansky (1951), who presents results of a mating selection experiment with *Drosophila pseudoobscura* and *D. persimilis,* states that when one is dealing with closely related but distinct species, though females of both species may be inseminated, there are more conspecific than interspecific fertilizations. Similar results were obtained by the senior author (Rozeboom, 1953) with the three species of *Anopheles.* In many of the replicates of the experiments with the *C. pipiens* populations, there were significantly higher percentages of cross inseminations than of inseminations between sexes within a given population. This would support our previous view that we are dealing, in the *C. pipiens* complex of North America, with subspecific populations rather than with species.

Although these experiments fail to demonstrate an ability on the part of these populations to recognize members of the opposite sex of their own kind, they do reveal that subtle differences exist in the biology of these populations, as exemplified by the differences in sexual activity. Under identical laboratory conditions, *C. molestus* was by far the most active, *C. pipiens* was the least, and *C. quinquefasciatus* was indermediate. A small difference was noted even between the two strains of *C. quinquefasciatus.* Evidently stimuli within the laboratory were lacking which would induce maximum mating responses on the part of *C. pipiens.* These stimuli must exist in the natural environment of *pipiens,* and it is conceivable that the different populations become sexually active under slightly different circumstances. Such a mechanism would tend to keep them at least partially separated from one

another, and this might be a means of preventing complete blending of the forms now recognizable in North America, in those areas where their ranges overlap.

Our preoccupation with the specific or subspecific status of these and other mosquito populations may seem to many entomologists to represent a considerable overemphasis of a minor point in taxonomy. Certainly it should not be difficult for taxonomists to come to an agreement as to whether to refer to *C. pipiens* and *quinquefasciatus* as species or subspecies. However, we are not concerned here with nomenclature. If one accepts as an hypothesis that species evolve gradually through accumulations of genetic changes, it becomes apparent that species complexes in the CULICIDAE represent opportunities for investigations which may lead to a better understanding of the mechanisms of speciation among these insects.

SUMMARY AND CONCLUSIONS

Mating experiments with *Culex pipiens, C. molestus,* and two strains of *C. quinquefasciatus* failed to demonstrate an ability on the part of the members of these populations to recognize the opposite sex of their own kind. Differences in insemination rates apparently were due to the varying degrees of sexual activity exhibited by these populations under identical laboratory conditions. Thus, subtle differences do exist in the biology of these populations, even between the two strains of *C. quinquefasciatus.* It is suggested that differential sexual activity may be a means whereby these populations could maintain their identity in nature, even in areas where their ranges overlap. However, it is felt that the results of the present experiments do not warrant the conclusion that *C. pipiens, quinquefasciatus,* and *molestus* in North America are of more than subspecific status.

REFERENCES

BARR, A. R. AND KARTMAN, L. 1951 Biometrical notes on the hybridization of *Culex pipiens* L. and *C. quinquefasciatus* Say. J. Parasit. **37:** 419–420.

BATES, M. 1949 The natural history of mosquitoes. The MacMillan Co., N. Y.: xv + 379 pp.

DOBZHANSKY, T. 1951 Genetics and the origin of species. Columbia Univ. Press, N. Y.: x + 364 pp.

FARID, M. A. 1949 Relationships between certain populations of *Culex pipiens* Linnaeus and *Culex quinquefasciatus* Say in the United States. Amer. J. Hyg. **49:** 83–100.

KNIGHT, K. L. 1953 Hybridization experiments with *Culex pipiens* and *C. quinquefasciatus* (Diptera, Culicidae). Mosq. News. **13:** 110–115.

MATTINGLY, P. F., ROZEBOOM, L. E., KNIGHT, K. L., LAVEN, H., DRUMMOND, F. H., CHRISTOPHERS, S. R., AND SHUTE, P. G. 1951 The *Culex pipiens* complex. Trans. Roy. Ent. Soc. London **102:** 331–382.

ROZEBOOM, L. E. 1953 Sexual isolation in some North American *Anopheles* mosquitoes. Amer. J. Trop. Med. Hyg. **2:** 677–682.

SUNDARARAMAN, S. 1949 Biometrical studies on integration in the genitalia of certain populations of *Culex pipiens* and *Culex quinquefasciatus* in the United States. Amer. J. Hyg. **50:** 307–314.

FOUR NEW SPECIES OF CESTODES OF RODENTS FROM THE HIGH PLAINS, CENTRAL AND SOUTHERN ROCKIES AND NOTES ON *CATENOTAENIA DENDRITICA*[1]

CHARLES F. SMITH[2]

Swift & Company, Research Laboratories, U. S. Yards, Chicago, Illinois

During the summers of 1948 and 1951, more than 895 small mammals collected from Kansas, Oklahoma, New Mexico, Colorado, Wyoming and South Dakota by field parties from the University of Kansas, Museum of Natural History, were examined for helminth parasites. Twenty-nine species of adult cestodes were recovered. Four of these species appear to be new to science and one of them belongs to a new genus.

The cestodes were killed and preserved in 5% formalin. Toto mounts were prepared according to Riser's (1950) modification of Proescher's Celestin Blue B stain.

Family ANOPLOCEPHALIDAE Fuhrmann, 1907

Subfamily ANOPLOCEPHALINAE Blanchard, 1891

Hymenandrya thomomyis n. gen., n. sp.

(Figs. 1 to 5)

Description (Based on 5 mature specimens): Length, 70 to 90 mm. Maximum width, at posterior end, 3.50 mm. Scolex cone-shaped, 0.35 to 0.45 mm. long by 0.27 to 0.30 mm. wide. Rostellum absent. Suckers circular, unarmed, 0.12 to 0.13 mm. in diameter; orifice measuring up to 0.05 mm.; musculature well developed. Neck, 0.90 to 1.50 mm. long. Genital pores, unilateral, dextral, in middle of anterior half of lateral margin of proglottid. Dorsal excretory vessels lateral to ventral excretory vessels and approximately 0.005 mm. in diameter.

Testes, 7 to 15 in number, ovoid in outline and with smooth margins, from 0.024 mm. in diameter to 0.11 mm. long by 0.05 mm. wide, divided by female complex into poral and aporal groups, with poral group varying from none (very rare) to 7 testes, most commonly 1 to 3 testes, and aporal group from 5 to 9, most commonly 6 to 8. Testes extending laterally past the excretory vessels. Cirrus sac, 0.19 to 0.22 mm. long by 0.02 to 0.03 mm. wide, passing dorsal to excretory vessels and extending to, but not past ventral excretory vessel. External and internal seminal vesicles present. Cirrus measuring up to 0.06 mm. long and with minute spines.

Ovary fan-shaped, lobed, close to anterior border of proglottid and slightly poral in location. Vagina extending dorsal to excretory vessels and lying posterior to cirrus sac; both opening through common genital pore. Proximal half of vagina enlarging to form seminal receptacle. Vitellarium ovoid, slightly lobed, posterior and dorsal to ovary. Uterus at first a transverse, reticulate structure in anterior portion of proglottid, sometimes overlapping excretory vessels on either side, aporal half developing more rapidly than poral half. In gravid proglottids, uterus not completely breaking down and becoming saccate, but retaining some of its reticulate nature, restricting distribution of eggs in proglottid.

Entire egg either spherical, measuring about 50 micra in diameter or, more often, ovoid in outline, 46 to 54 micra long by 18 to 40 micra wide. Onchosphere with three coverings, outer shell a thin membrane, middle membrane separated from embryophore by a space filled with jelly-like material having a granular appearance. Embryophore approximately 1.3 micra thick with a slender process (approximately 0.013 mm. long) at either end, doubtless representing prongs of a "pyriform apparatus" characteristic of other anoplocephalids. Onchosphere, 13 to 16 micra long by 6.5 to 10 micra wide.

Host: Thomomys talpoides ssp., northern pocket gopher.

Location: Small intestine.

Locality: 5 miles North and 22 miles West of Saguache, 10,000′, Saguache County, Colorado.

Received for publication, September 1, 1953.

[1] Studies from the Department of Zoology, University of Nebraska, No. 265. The work was done under the direction of Dr. H. W. Manter.

[2] Formerly at the University of Nebraska.

Cotype specimens: U.S. National Museum, Helm. Coll. No. 49065.

Generic diagnosis: Anoplocephalinae, *Hymenandrya,* of medium size. Scolex without rostellum or armature. Vagina and sperm duct crossing excretory canals dorsally. Seven to 15 testes divided by ovary and vitellarium into aporal and poral groups, rarely contiguous. Cirrus sac elongated, extending inward to ventral excretory vessel. Vagina opening posterior to cirrus sac through common genital pore. Ovary in anterior half of proglottid and slightly poral in location. Uterus a transverse reticular network not completely disappearing in gravid proglottids, overlapping excretory vessels. Ova with 3 coverings; pyriform apparatus of embryophore in form of 2 polar extensions, 1 arising from each end.

Type species: Hymenandrya thomomyis. The name *Hymenandrya* indicates similarities to both *Hymenolepis* and *Andrya.*

Discussion: The reticulate uterus and pyriform apparatus are anoplocephaline in character. The testicular arrangement is like that of Hymenolepididae. The embryophore simulates a combination of the position of the filaments of the hymenolepids with the structure of the pyriform apparatus of the anoplocephalids. *Hymenandrya* more closely resembles the genus *Andrya* in possessing a reticulate uterus, vagina posterior to cirrus pouch, and pyriform-like apparatus. It can be most readily differentiated from *Andrya* by the fewer number of the testes and their position, and by the atypical pyriform apparatus.

Hymenandrya shows some resemblance to *Chitinolepis* Baylis, 1926 and *Pseudoligorchis* Johri, 1934, genera created for a single species each, *C. mjoebergi* Baylis, 1926 and *P. magnireceptaculata* Johri, 1934 respectively, and placed in the family Hymenolepididae, subfamily Hymenolepidinae. Wardle and McLeod (1952) noted that these genera have certain characters which are unusual for the family Hymenolepididae, making their true relationships somewhat doubtful. *C. mjoebergi* came from a rat, *Rattus sabanus,* in Sarawak, East Indies. The arrangement of its genital organs resembles that of *H. thomomyis,* and a mass of material between the embryophore and outer membrane was noted by Baylis. The species differs from *H. thomomyis* in having a rudimentary rostellum, 9 to 12 testes in a transverse row, a transverse sac-like gravid uterus which is as wide as the segment, and a thick egg shell. *P. magnireceptaculata,* a bat parasite from Lucknow, India, has the genital organs arranged similarly to *H. thomomyis.* It differs from *H. thomomyis* in having a rostellar sac, 8 to 12 testes, genital ducts passing between the poral excretory canals, and an irregularly lobed uterine sac.

Some characters such as shape and size of eggs and early uterine development are omitted from the original descriptions of *C. mjoebergi* and *P. magnireceptacula.* A re-examination of material of *Chitinolepis* and *Pseudoligorchis* might show that they are anoplocephalids near the genus *Hymenandrya.*

Family DAVAINEIDAE Fuhrmann, 1907
Subfamily DAVAINEINAE Braun, 1900
Raillietina (Raillietina) sigmodontis n. sp.
(Figs. 6 to 12)

Description (Based on 9 specimens): Length, 80 mm. Maximum width, 2 mm. Scolex, 0.35 to 0.44 mm. long by 0.23 to 0.36 mm. wide. Rostellum poorly developed, with 66 hooks in two rows. Hooks (Fig. 7), hammer-shaped, 0.020 to 0.022 mm. long, anterior root and blade 0.013 mm. across. Rostellar sac cup-shaped, 0.10 mm. in diameter. Diameter of rostellum at crown of hooks, 0.09 mm. Suckers, 0.10 to 0.11 mm. in diameter, deeply cup-shaped with aperture approximately 0.06 mm. in diameter. Edges of suckers covered with small spines approximately 2 micra long.

Neck, 0.08 to 3.00 mm. long. Mature segments 1.50 mm. wide by 0.25 mm. long, but segments with developing egg capsules 1.20 mm. wide by 0.50 mm. long. Gravid segments sometimes becoming longer than wide. Ventral excretory vessels conspicuous (0.03 mm. in

diameter). Dorsal excretory vessels inconspicuous. Genital pores unilateral in position, situated in the anterior third of segment.

Cirrus sac, 0.13 to 0.15 mm. long by 0.04 to 0.05 mm. wide, extending to, but not past, the ventral excretory vessel. Cirrus unarmed, as much as 0.03 mm. long. Testes approximately 0.04 mm. in diameter in fully mature segments, approximately 17 in number, divided by the female complex into poral and aporal groups; poral group numbering approximately five and aporal group approximately twelve.

Ovary and vitellaria slightly poral in position. Ovary lobed and in anterior portion of proglottid. Vitellaria posterior and slightly aporal to ovary. Vagina posterior to vas deferens, with muscular wall taking stain deeply as far as excretory vessels, then becoming widened with thin walls and serving as a seminal receptacle.

30 to 35 egg capsules in the gravid segment, each containing from 15 to 25 eggs. Egg capsules, 0.22 to 0.25 mm. long by 0.14 to 0.16 mm. wide. Egg measuring 24 micra in diameter; onchosphere 16 micra.

Host: Sigmodon hispidus texianus (Audubon and Bockman), Texas cotton rat.
Location: Small intestine.
Locality: 8 miles North of Driftwood, Alfalfa County, Oklahoma.
Cotype specimens: U.S. National Museum, Helm. Coll. No. 49062.

Discussion: Joyeux and Baer (1936, p. 615) gave in tabular form the characters for some *Raillietina* species of rodents from localities other than the North American continent. *R. sigmodontis* can be easily differentiated from these *Raillietina* by characters such as numbers of hooks and testes, size and shape of cirrus sac, number of egg capsules per segment and number of eggs per capsule. The only species of *Raillietina* recorded from North American rodents (excluding lagomorphs) is *R. (R.) bakeri* Chandler, 1942 from the tree squirrel *Sciurus niger rufiventer.* Although specimens of *R. bakeri* were available, they were unfavorable for comparison because of immaturity. Certain differential characters were observed between the two batches of specimens.

The following are some of the characters of *R. bakeri* and *R. sigmodontis* respectively: Rostellar hooks; 66, 66. Length of hooks; 20 to 22 micra, 20 to 22 micra. Number of testes; 30 to 40, 15 to 19. Size of cirrus pouch; 90 to 95 by 35 micra, 140 by 46 micra. Egg capsules per segment; 80 to 90, 30 to 35. Eggs per egg capsule; 6 to 9 (10), 15 to 25.

Cameron and Reesal (1951) and Stunkard (1953) have noted in *Raillietina demerariensis,* "considerable variation in dimensions and number of hooks, testes, and egg capsules even within tapeworms from the same host." *R. bakeri* has been reported by Melvin and Chandler (1950) from the cotton rat, *Sigmodon hispidus,* but no variations were described. On the absence of data on variations in *R. bakeri,* it seems wisest to consider *R. sigmodontis* a new species.

Family Catenotaeniidae Wardle and McLeod, 1952

Genus *Catenotaenia* Janicki, 1904

Specimens of what appeared to be *Catenotaenia dendritica* (Goeze, 1782) Janicki, 1904 were collected from one of ten sage brush voles, *Lagurus curtatus levidensis* (Goldman) taken 33 miles South and 22 miles East of Rock Springs, 7025′, Sweetwater County, Wyoming. As evidenced by their small size (30 mm. long) and by the presence of an apical organ in one specimen (Fig. 13) the material represented young mature worms. Joyeux and Baer (1945) showed that the apical organ disappeared with maturity, and indicated that *Catenotaenia ris* Yamaguti, 1942 was a synonym of *C. dendritica.*

In the original description of *C. linsdalei,* based on two fragmented specimens

without scolices, McIntosh (1941) did not give the distinctions between *C. linsdalei* and *C. dendritica,* although he stated that *C. linsdalei* had (1) 40 to 50 lateral uterine branches, (2) an average number of 130 testes, and (3) small eggs measuring 6 to 7 micra. McIntosh's figure (p. 61) of a gravid proglottid showed 50 lateral uterine branches only when the most lateral tips were counted. The number of primary uterine branches must have been smaller than stated, thus probably corresponding with the number of uterine branches in *C. dendritica.*

In examining the original material described as *C. dendritica* by Riggenbach (1895), Joyeux and Baer (1945) found that the number of testes was 140 to 190 (usually 150 to 160) rather than the 200 to 210 given by Riggenbach. Voge (1948) reported *C. linsdalei* from kangaroo rats (*Dipodomys venustrus* and *D. heermanni*) and pocket mice (*Perognathus californicus*) in California, stating that the number of testes ranged from 90 to 150 (0.034 to 0.085 mm. in diameter) and that the cirrus sac ranged from 0.127 to 0.170 mm. in length.

These additional facts concerning the two species result in a closer similarity between *C. linsdalei* and *C. dendritica.* In view of these considerations, I consider *C. linsdalei* a synonym of *C. dendritica.*

Catenotaenia dendritica has been recorded from squirrels (*Sciurus vulgaris* L., *Sciurus ris* Temm., and *Sciurus* sp.) in Eurasia and Africa and *C. linsdalei* (= *C. dendritica*) from the pocket gopher (*Thomomys bottae bottae*), kangaroo rats (*Dipodomys venustus* and *D. heermanni*) and pocket mice (*Perognathus californicus*) in California. The recording of *C. dendritica* from the sage brush vole, *Lagurus curtatus levidensis,* adds a new host record and extends the range of the parasite in North America. The specimens from the sage brush vole are deposited in the U.S. National Museum, Helm. Coll. No. 49055.

Catenotaenia peromysci n. sp.

(Figs. 14 to 17)

Description (Based on 5 specimens): Length, 65 mm. Width, 1.20 to 1.70 mm. Scolex, 0.33 to 0.36 mm. wide. Suckers, 0.09 to 0.12 mm. in diameter. Apical organ not observed. Neck approximately 0.40 mm. long. Proglottids (relaxed, maximum size): mature, 1.20 mm. long by 0.90 mm. wide; gravid, 2.60 mm. long by 1.40 mm. wide. Genital pores lateral, in anterior half of proglottid, irregularly alternating.

Testes in 1 group, posterior to ovary, between excretory vessels, 70 to 80 in number, subspherical 0.072 by 0.054 mm. at maximum size. Cirrus sac, 0.16 to 0.22 mm. by 0.05 to 0.07 mm.

Ovary deeply lobed, in anterior half of proglottid, mostly on the aporal side. Vitellarium lobed, in poral half of proglottid, level with posterior half of ovary. Vagina extending transversely, parallel with male duct, curving posteriorly at middle of proglottid, entering female gland complex; at times enlarging to form seminal receptacle. Shell gland anterior to vitellarium. Uterus extending longitudinally in median portion of proglottid; 25 to 30 primary lateral uterine branches on each side. Entire egg, 39 by 13 micra; embryophore, 13 to 16 micra by 8 to 9 micra; onchosphere, 11 by 6 to 7 micra.

Host: Peromyscus maniculatus rufinus (Merriam), deer mouse.

Location: Small intestine.

Localities: 2 miles South and 4 miles West of Coyote, 8100′ (type locality), Rio Arriba County, New Mexico; 28 miles North and 5 miles West of Mack, 7250′, Mesa County, in Garfield County, Colorado; 32 miles South and 22 miles East of Rock Springs, 7025′, Sweetwater County, Wyoming; 3 miles North and 5 miles East of Orin, 4725′, Converse County, Wyoming; 2 miles South of Pine Bluffs, 5200′, Laramie County, Wyoming.

Cotype specimens: U.S. National Museum, Helm. Coll. No. 49063.

Discussion: This species appears to be closely related to *Catenotaenia pusilla* (Goeze, 1782). *C. pusilla* has been recorded from a variety of rodent hosts in Europe, North America and Japan. These hosts are *Mus musculus* L., *Mus wageneri*

albellus Kishida, *Apodemus sylvaticus* L., *Rattus rattus* L., *Rattus norvegicus* (Erxl.), *Evotomys glareolus* Schrebl., *Microtus agrestis* L., and *Microtus arvalis* Pall.

In general, the various structures of *C. peromysci* are larger than those of *C. pusilla*. *C. peromysci* has the larger portion of the ovary extending longitudinally in the aporal half of the proglottid, and 25 or more primary uterine branches on each side. In contrast, *C. pusilla* has the ovary centrally located in the anterior half of the segment, and 9 to 13 uterine branches on each side.

Catenotaenia laguri n. sp.

(Figs. 18 to 22)

Description (Based on 10 specimens): Strobila long, thin, delicate. Length, 30 to 50 mm. Width, 0.70 mm. Scolex, 0.22 to 0.26 mm. wide. Suckers, 0.09 to 0.10 mm. in diameter. Structure on apical end of one scolex, faintly visible, resembling apical organ. Neck 0.90 to 1.20 mm. long. Proglottids (relaxed, maximum size): mature, 1.60 mm. long by 0.66 mm. wide; gravid, 3.50 mm. long by 0.53 mm. wide. Genital pores lateral, in anterior 3/8 of proglottid, alternating irregularly.

Testes subspherical, 35 to 40 in number, in each of 2 lateral groups, contiguous posteriorly, maximum size 0.05 mm. Cirrus sac, 0.16 mm. long by 0.08 mm. wide.

Ovary deeply lobed, in anterior aporal half of proglottid. Vitellarium lobed, in poral half of proglottid, level with posterior half of ovary. Vagina a sinuous, rather than straight tube, not paralleling male duct, extending immediately posteriorly, enlarging at times to form seminal receptacle. Shell gland anterior to vitellarium. Uterus longitudinal and median; 35 to 40 primary lateral uterine branches on each side. Entire egg, 62 by 60 micra; embryophore, 20 to 24 by 20 micra; onchosphere 14 to 20 micra by 10 to 16 micra.

Host: Lagurus curtatus levidensis Goldman, sage brush vole.

Location: Small intestine.

Locality: 32 miles South and 22 miles East of Rock Springs, 7025′, Sweetwater County, Wyoming.

Cotype specimens: U.S. National Museum, Helm. Coll. No. 49064.

Discussion: C. laguri appears to be closely related to *C. geosciuri* Ortlepp, 1938, which has been recorded from *Geosciurus capensis* Kerr, *Xerus rutilus stephanson* Thomas, and *Euxerus erythropus* E. Geoff. in Africa. In these two cestode species, the vagina does not parallel the male duct. *C. laguri* differs from *C. geosciuri* by

TABLE 1.—*Characters of some species of* Catenotaenia *of rodents. Measurements in mm.*

Species	*Catenotaenia peromysci*	*Catenotaenia pusilla*	*Catenotaenia geosciuri*	*Catenotaenia laguri*	*Catenotaenia reggiae*
Authority	Author	Joyeux and Baer, 1945	Joyeux and Baer, 1945	Author	Rausch, 1951
Length	65	30 to 160	93	30 to 50	360
Width	1.2 to 1.7	0.75 to 1.65	1.6	0.7	3
Scolex	0.33 to 0.36	0.23 to 0.30	0.27 to 0.35	0.24 to 0.26	0.36 to 0.44
Testes Position	One group	One group	One group	Two groups totaling	Two groups totaling
Testes Number	70 to 80	70 to 100	200	70 to 80	300 or more
Cirrus sac L	0.16 to 0.22	0.14 to 0.18	0.20 to 0.26	0.16 to 0.18	0.32 to 0.49
Cirrus sac W	by 0.05 to 0.07	by 0.07 to 0.09	by 0.11 to 0.13	by 0.08 to 0.09	by 0.14 to 0.19
Primary Uterine Branches	25 to 30	9 to 13	12 to 18	35 to 40	30 to 40
Eggs	0.039 to 0.013	0.022 to 0.012	0.023 to 0.025 by 0.014	0.060 by 0.062	0.017 to 0.028
Embryophore	0.013 to 0.016 by 0.009			0.02 to 0.024 by 0.02	
Onchosphere L	0.011 to 0.012	0.014	0.014 to 0.015	0.014 to 0.020	
Onchosphere W	by 0.006 to 0.007	by 0.010	by 0.011	by 0.010 to 0.016	

possessing 35 to 40 testes in each of 2 groups, rather than 200 testes in 1 group and by having 35 to 40 primary uterine branches rather than 12 to 18. *C. laguri* also shows some similarities (two groups of testes; 35 to 40 primary uterine branches), to *C. reggiae* Rausch, 1951, from the arctic marmot, *Marmota caligata broweri* Hall and Gilmore, of Alaska. *C. laguri* differs from *C. reggiae* by possessing 35 to 40 testes in each of two groups rather than 150 and by being much smaller.

The distinguishing characters of the two new *Catenotaenia* species described above and the species they resemble are shown in Table 1.

SUMMARY

One new genus and 4 new species of cestodes are named and described, viz. *Hymenandrya thomomyis* (ANOPLOCEPHALIDAE) from the pocket gopher, *Thomomys talpoides* ssp., of Colorado; *Raillietina* (*Raillietina*) *sigmodontis* (DAVAINEIDAE) from the cotton rat, *Sigmodon hispidus texianus,* of Oklahoma; *Catenotaenia peromysci* (CATENOTAENIIDAE) from the deer mouse, *Peromyscus maniculatus rufinus,* of New Mexico, Colorado and Wyoming; and *Catenotaenia laguri* from the sage brush vole, *Lagurus curtatus levidensis,* of Wyoming.

Catenotaenia dendritica (Goeze, 1782) Janicki, 1904 is reported from the sage brush vole, *Lagurus curtatus levidensis* Goldman from Wyoming, and it is suggested that *Catenotaenia linsdalei* McIntosh, 1941 be considered a synonym of *C. dendritica.*

REFERENCES

BAYLIS, H. A. 1926 Some parasitic worms from Sarawak. Sarawak Museum J. **3**: 303–322.

CAMERON, T. W. M. AND REESAL, M. R. 1951 Studies on the endoparasitic fauna of Trinidad mammals. VII. Parasites of hystricomorph rodents. Canad. J. Zool. **29**: 276–289.

CHANDLER, A. C. 1942 Helminths of tree squirrels in southeast Texas. J. Parasit. **28**: 135–140.

JOHRI, L. N. 1934 Report on a collection of cestodes from Lucknow (U.P., India). Rec. Indian Mus. **36**: 153–177.

JOYEUX, C. AND BAER, J. G. 1936 Helminths des rats de Madagascar. Contribution à l'étude de *Davainea madagascariensis* (Dav., 1869). Bull. Soc. Path. Exot. **29**: 611–619.

———— AND ————1945 Morphologie, évolution et position systématique de *Catenotaenia pusilla* (Goeze, 1782) cestode parasite de rongeurs. Rev. Suisse Zool. **52**: 13–51.

MCINTOSH, A. 1941 A new dilepidid cestode, *Catenotaenia linsdalei,* from a pocket gopher in California. Proc. Helm. Soc. Wash. **8**: 60–62.

MELVIN, D. M. AND CHANDLER, A. C. 1950 New helminth records from the cotton rat *Sigmodon hispidus,* including a new species *Strongyloides sigmodontis.* J. Parasit. **36**: 505–510.

RAUSCH, R. 1951 Studies on the helminth fauna of Alaska. VII—On some helminths from arctic marmots with the description of *Catenotaenia reggiae* n. sp. (Cestoda: Anoplocephalidae). J. Parasit. **37**: 415–418.

RISER, N. W. 1950 Notes on toto-mount technique. Proc. Helm. Soc. Wash. **17**: 132–133.

STUNKARD, H. W. 1953 *Raillietina demerariensis* (Cestoda), from *Proechimys cayennensis trinitatus* of Venezuela. J. Parasit. **39**: 272–279.

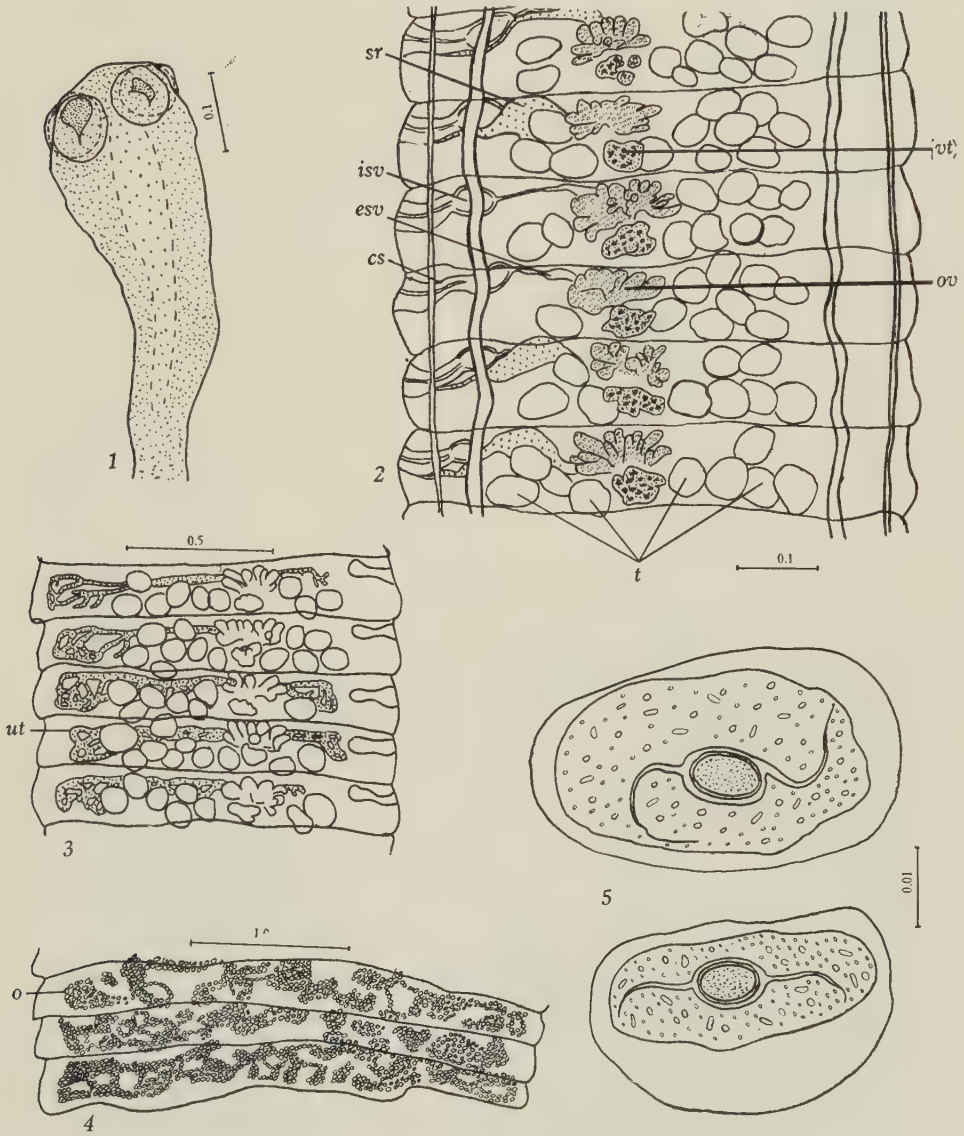
VOGE, M. 1949 New rodent hosts for *Catenotaenia linsdalei* with an additional description of this cestode. Trans. Amer. Micro. Soc. **67**: 266–267.

WARDLE, R. A. AND MCLEOD, J. A. 1952 The zoology of tapeworms. Uni. of Minn. Press. Minneapolis, 780 pp.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida, minor details and corrections being supplied freehand. Abbreviations: *ao,* apical organ; *cs,* cirrus sac; *ded,* dorsal excretory duct; *ec,* egg capsule; *esv,* external seminal vesicle; *exv,* excretory vessel; *isv,* internal seminal vesicle; *o,* ovum; *ov,* ovary; *sg,* shell gland; *sr,* seminal receptacle; *t,* testes; *ut,* uterus; *ved,* ventral excretory duct; *vt,* vitellarium.

PLATE I



Hymenandrya thomomyis

FIG. 1. Scolex.

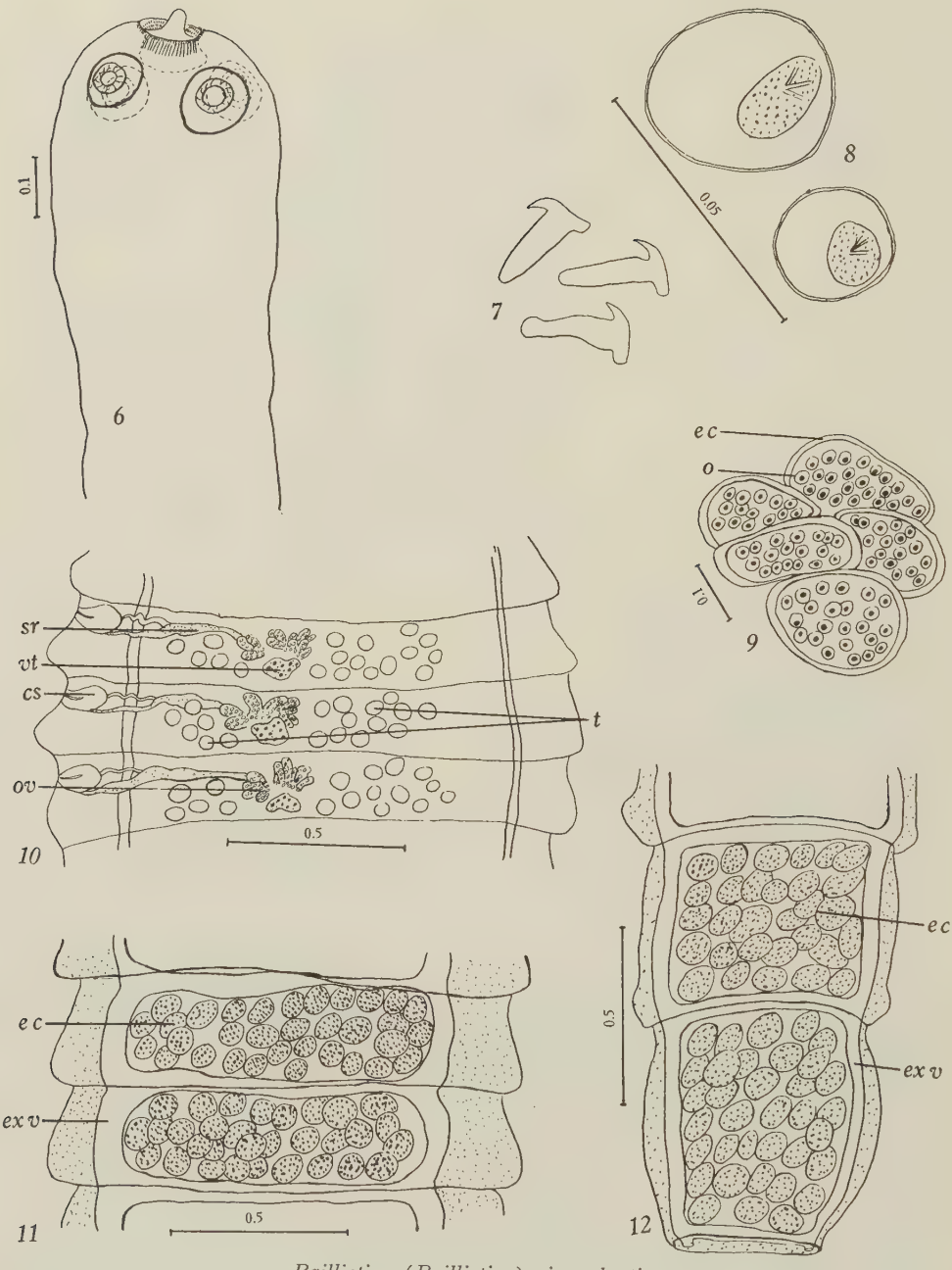
FIG. 2. Mature proglottids, ventral view. Vagina and seminal receptacle not shown in every segment in order that the vas deferens and external seminal vesicle may be shown.

FIG. 3. Young gravid proglottids with developing reticulate uteri.

FIG. 4. Gravid, terminal segments showing restricted distribution of eggs. Scale equals 1.0 mm.

FIG. 5. Eggs, showing the shape of the embryophore and the middle mass of material between the embryophore and outer shell.

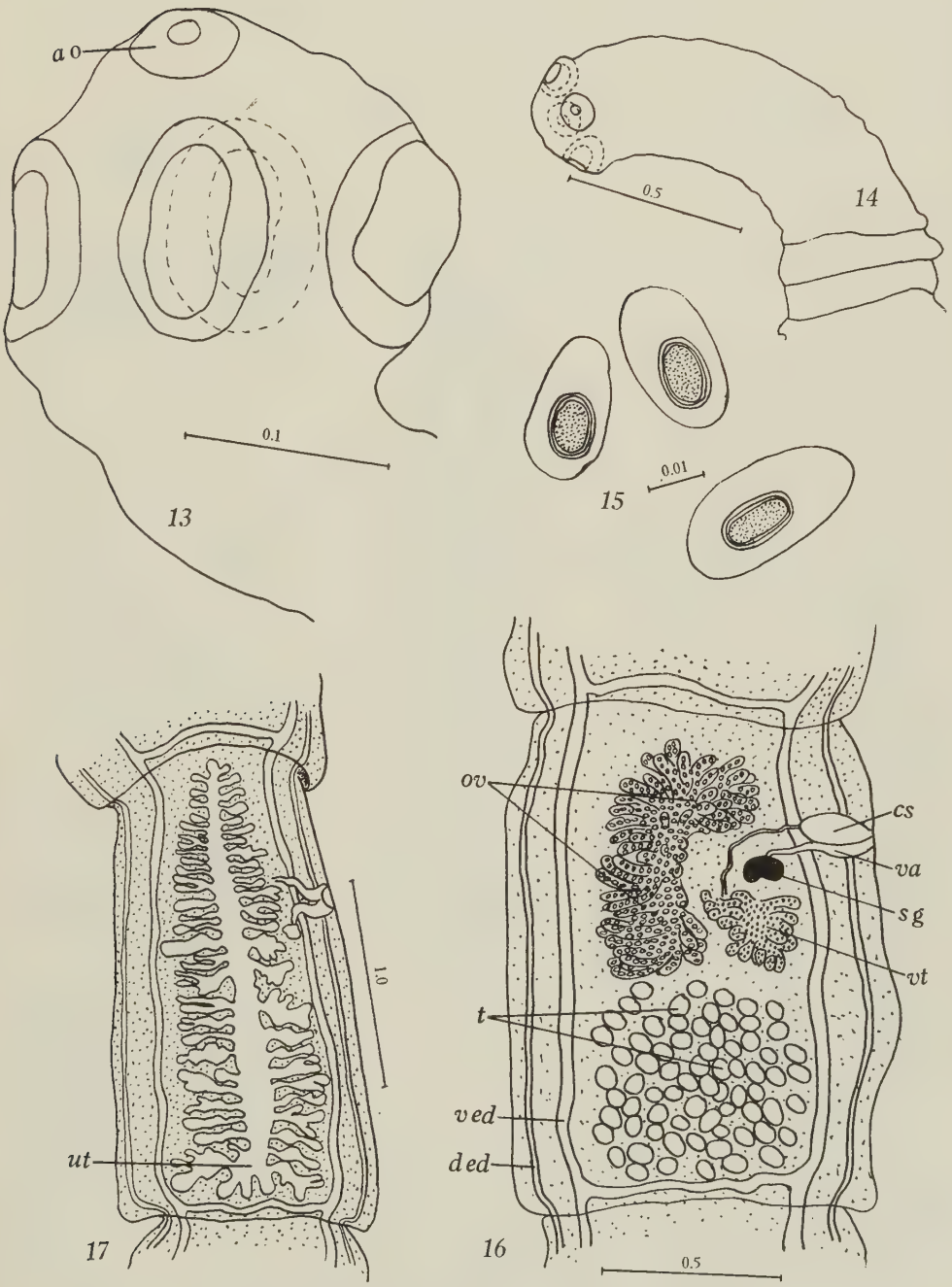
PLATE II



Raillietina (Raillietina) sigmodontis

FIG. 6. Scolex.
FIG. 7. Rostellar hooks.
FIG. 8. Eggs.
FIG. 9. Ova within egg capsules.
FIG. 10. Mature proglottids, dorsal view.
FIG. 11. Young gravid proglottids with developing egg capsules.
FIG. 12. Gravid terminal proglottids with egg capsules.

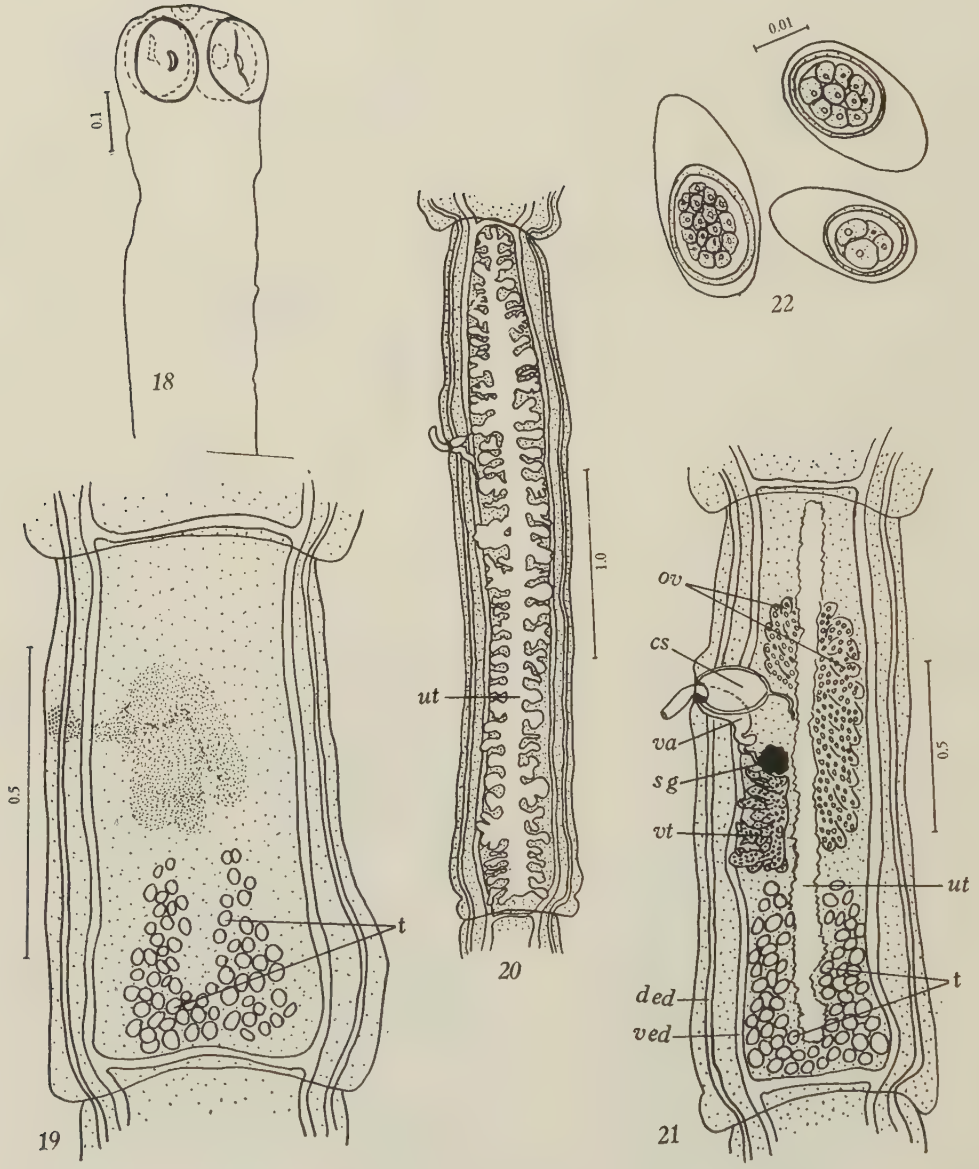
PLATE III



Catenotaenia dendritica and *Catenotaenia peromysci*

FIG. 13. Scolex of *C. dendritica* showing apical organ.
FIG. 14. Scolex of *C. peromysci.*
FIG. 15. Eggs of *C. peromysci* showing outer membrane.
FIG. 16. Mature proglottid, dorsal view, of *C. peromysci.*
FIG. 17. Gravid proglottid, showing uterus and uterine branching of *C. peromysci.*

PLATE IV



Catenotaenia laguri

FIG. 18. Scolex.
FIG. 19. Young maturing proglottid, ventral view.
FIG. 20. Fully gravid proglottid, showing uterus and uterine branching.
FIG. 21. Mature proglottid, dorsal view, showing early development of uterus.
FIG. 22. Eggs with outer membrane, showing different cellular stages of developing embryo.

STUDIES ON THE LIFE HISTORY OF *POSTHODIPLOSTOMUM MINIMUM* (MacCALLUM 1921)

JOSEPH HENRY MILLER

University College, New York University

INTRODUCTION

Complete life histories in the STRIGEIDA are few and the result of comparatively recent investigations. Previous to 1920, the developmental stages of these trematodes were neglected by helminthologists, who were diverted by Leuckart's (1889) theory that the holostomes were "metastatic" trematodes having an alternation of hosts but not of generations. The basis for the theory was the superficial resemblance of developing holostome miracidia to tetracotyles found in nature. This similarity led Leuckart to the belief that tetracotyles develop directly from ova without the interpolation of a cercarial stage. Lutz (1921) showed that the tetracotyle of *Cotylurus cornutus* in *Planorbis sp.* developed from a furcocercous cercaria. This experimental demonstration and its confirmation by Mathias (1922) and Ruszkowski (1922) invalidated the "metastatic" theory and investigators turned with renewed interest to a study of the STRIGEIDA. The most intensive investigations in North America were carried out at the University of Michigan by La Rue and his students in the period since 1926.

The first record of *Posthodiplostomum minimum* was made by Leidy (1856), who found the metacercaria encysted in the liver of a sunfish *Lepomis sp.* He erroneously identified this larval stage as *Diplostomum cuticola* von Nordmann, 1832, a European form encysted in the integument of fresh-water fish. Mac Callum (1921) inadequately described the adult from *Ardea herodias* (great blue heron) which died at the New York Zoological Park. He named this adult *Diplostomum minimum.* Agersborg (1926) described a metacercaria from *Hyborhynchus notatus* (blunt nosed minnow) which he named *Diplostomum van cleavei.* Hughes (1928) considered Agerborg's metacercaria identical with the metacercaria reported by Leidy (1856).

Various strigeid larval types have been categorized as an aid to the recognition and description of morphologically similar larvae. Hughes (1927) erected the larval group Neascus to contain the metacercaria *Neascus ambloplitis* nov. sp. from a sunfish (= *Uvulifer ambloplitis* according to Hunter and Hunter, 1935) and *Neascus cuticola* (= *Diplostomum cuticola* von Nordmann, 1832). Recognized larval types were: TETRACOTYLE Filippi, 1854, in which the body is pyriform, with or without a small hind-body, with well developed lateral suckers, and with the reserve bladder in the form of large spaces; CODONOCEPHALUS Diesing, 1850, fore-body reduced to a flattened disc, lateral suckers absent, excretory system a complicated

Received for publication, October 2, 1953.

A part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Arts and Science, New York University.

The writer wishes to express his gratitude to Professor Horace W. Stunkard for his encouragement and guidance in the pursuit of this problem and for his patient and scholarly criticism in the preparation of the manuscript.

Present address: Louisiana State University School of Medicine, Department of Microbiology, New Orleans, Louisiana.

net work of two groups of vessels; NEASCUS Hughes, 1927, fore- and hind-body well developed and separated by a constriction, no lateral suckers, reserve bladder well developed; and DIPLOSTOMULUM Brandes, 1892 [revised by Hughes (1929), to include *Diplostomum* von Nordmann, 1832 and *Tylodelphys* Diesing, 1850], hind-body reduced to a conical prominence, reserve bladder a more or less definite arrangement of tubules, usually a pair of lateral suckers on the fore-body.

Hughes (1928) redescribed *Diplostomum van cleavei,* since Agersborg had confused anterior and posterior ends and made other comparable errors in his description. Hughes included this form in his larval group Neascus, renaming it *Neascus van cleavei.*

Dubois (1937) erected the larval group POSTHODIPLOSTOMULUM to contain *N. van cleavei* (Agersborg, 1926), *Neascus oneidensis* Van Cleave and Muller, 1932 (syn. *Neascus van cleavei* according to Van Cleave and Mueller, 1934) and *Neascus grandis* Van Cleave and Mueller, 1932. Dubois justified this grouping since *Neascus ambloplitis* Hughes, 1927, type of the group NEASCUS, was shown by Hunter and Hunter (1935) to be the metacercaria of a species in the subfamily CRASSIPHIALINI Dubois, 1936.

Because of Mac Callum's inadequate description of the adult *Diplostomum minimum,* Noble (1936) erroneously described the same species as *Neodiplostomum orchilongum* from the heron, *Ardea herodias,* in California.

Dubois (1936) erected the genus *Posthodiplostomum* to contain certain members of the DIPLOSTOMIDAE parasitic in the ARDEIDAE. *Diplostomum minimum* was included in this new genus as *Posthodiplostomum minimum* (Mac Callum, 1921). Ferguson (1937) considered *N. orchilongum* a synonym of *P. minimum* and Dubois (1938) in his monographic study of the Strigeida, listed *N. orchilongum* Noble, 1936 as a synonym of *P. minimum.*

Dubois and Rausch (1948) made additions to the description of *P. minimum* from specimens collected by Rausch from *Nycticorax nycticorax* (black-crowned night heron) and *Ardea herodias.*

Prior to the present account, two cercariae have been presented as the larval stage of *P. minimum,* namely *Cercaria multicellulata* and *Cercaria louisiana.*

H. M. Miller (1923, 1925) described a furcocercous pharyngeal monostome cercaria, *C. multicellulata,* from *Physa gyrina* (= *Physella gyrina*) in Illinois.

E. L. Miller (1935, 1936) described *C. louisiana,* a furcocercous pharyngeal monostome cercaria from the same host, collected in Illinois and Louisiana. E. L. Miller's two descriptions do not agree on the shape of the cercaria when at rest, lack specific characteristics for the differentiation of the species, and present erroneous interpretations of certain structures. This cercaria is considered "species indeterminata" by the writer.

Hunter (1936), working in New York, reported the penetration of *Lepomis gibbosus* (common sunfish) by cercariae identified as *C. multicellulata* from the snail, *Physa gyrina.* The metacercariae, encysted in the viscera, were identified as *Neascus van cleavei.*

Ferguson (1936, 1937, 1938), working in Illinois, reported the life history of *Posthodiplostomum minimum.* Specimens of *Hyborhynchus notatus* (blunt-nosed minnow) were exposed to cercariae identified as *C. multicellulata* from the snail, *Physa integra.* Subsequent examination of the minnows showed metacercariae en-

cysted in the liver, kidneys and other organs. Ferguson's controls, however, showed that all minnows used in the experiment harbored natural infections with Neascus type metacercariae. Adult strigeids were obtained by feeding encysted metacercariae from minnows exposed to *C. multicellulata* and from naturally infected minnows and sunfish to day-old chicks. Ferguson believed that the metacercariae were identical with *Neascus van cleavei* and stated that the adults which matured in the chicks were "similar" to *P. minimum* in naturally infected herons. Hunter (1937) and Hunter and Hunter (1940) studied the development of cercariae from *Physa gyrina* to encysted metacercariae in the common sunfish, *Lepomis gibbosus*. The cercariae were identified by them as *C. multicellulata*. The metacercariae were determined to be identical with *N. van cleavei*. They fed naturally infected *Hyborhynchus notatus* and *Helioperca macrochira* (blue-gill sunfish) to herons and obtained adult worms which they regarded as identical with specimens from naturally infected herons. These adults were determined to be members of the same genus as the adult worms reported by Ferguson (1937). Encysted metacercariae of the Neascus type, collected in the United States and Canada from twenty-three species of fish distributed over six families, were reported by Hunter and Hunter (1940) to represent the last larval stage of *Posthodiplostomum minimum*.

Ferguson (1940) cultured *Neascus van cleavei* from *Lepomis gibbosus* to mature *P. minimum* in bacteria-free chick serum plus yeast extract. The eggs produced by the adults, however, were abnormal and failed to hatch.

Klak (1939) reported the first indication of host specificity in the larval group Neascus. He found *Micropterus salmoides* and *Micropterus dolomieu* (large and small-mouth bass, respectively, fam. CENTRARCHIDAE) and *Notemigonus chrysoleucas* (golden-shiner, fam. CYPRINIDAE) uninfected with metacercariae in a pond where *Pimephales promelas, Hyborhynchus notatus, Notropis hudsonius* and *Notropis amoenus* (black-head, blunt-nosed, spot-tailed and attractive minnows respectively, fam. CYPRINIDAE) were infected with metacercariae, identified as *Neascus sp.*, which subsequent authors have considered identical with *N. van cleavei*. This finding was confusing, since *N. van cleavei* had been reported as a common parasite of both families of fish.

In an attempt to clarify Klak's observations, Ferguson (1943) fed metacercariae, believed to be those of *P. minimum*, from *Lepomis gibbosus* (CENTRARCHIDAE) and *Pimephelas promelas* (CYPRINIDAE), to two groups of trematode-free *Nycticorax nycticorax*. Laboratory raised *Physa acuta* and *Physa heterostropha* were exposed to the miracidia obtained from hatching the eggs of the adult strigeids developing in the herons. Both species of snails became infected and shed cercariae. Experimentally, two lines had been produced: one whose metacercariae were in fish of the CYPRINIDAE, and the other in which the CENTRARCHIDAE served as hosts. In penetration experiments with the two lines of cercariae, it was found that the cross-infection of the two families of fish was impossible; that is, the cercariae of the centrarchid line infected only members of that family, while those of the cyprinid line infected only the CYPRINIDAE. It would seem that the experiments of Ferguson confirm the observations of Klak; however, Ferguson stated that the fish ". . . used in the experiments all contained a small number of mature cysts of the *N. van cleavei* type. . . ." He also stated, "It was impossible to study critically the mor-

phology of the cercariae representing the minnow and sunfish lines. . . ." Further, he did not report a study of the adults developing in the two groups of herons.

Brooks (1948) reduced *Cercaria louisiana* to synonymy with cercaria *Posthodiplostomum minimum.* This systematic change was based on Hoffman's (1950) unpublished report of the development of *C. louisiana* to *Neascus van cleavei* in centrarchids. In the same report, Hoffman listed seventy-seven species of fish from fifteen families previously reported to harbor metacercariae identified as *N. van cleavei.*

From the preceding review of the literature concerning *P. minimum* and its developmental stages, it seems probable that there is a confusion of at least two species of strigeids whose larval and perhaps even adult stages are very similar. Ferguson (1943) summarized the situation aptly in the conclusion of his paper: "... it becomes apparent that the life cycle relationships of Miller's (1923) cercariae are unknown. The minnow origin of Agersborg's (1926) metacercariae is certain. In view of the uncertainties as to the specific status of the metacercariae now considered to those of *P. minimum,* it is evident that this confusion must be eliminated by morphological and experimental studies before the specific status of the trematodes in question can be determined."

During the spring of 1951, in a pond in the New York Botanical Gardens, the writer found a 100% infection of *Lepomis gibbosus* and *Lepomis megalotus* (pumpkin-seed sunfish) with metacercariae identified as *N. van cleavei.* The metacercariae occurred encysted in the heart, liver, kidneys, spleen, gonads and on the surface of the stomach, small intestine, peritoneum and mesenteries. Furcocercous monostome cercariae from *Physa heterostropha* collected in the same pond were proved experimentally to be the source of the infection in the sunfish. The morphology of the cercariae differed from previous descriptions of furcocercous monostome types in the literature, especially those of *Cercaria louisiana* and *Cercaria multicellulata,* reported as larval stages of *P. minimum.* Because of this morphological difference, a developmental study was undertaken to determine the relation of this cercaria to *P. minimum* and to clarify the latter's life history.

MATERIALS AND METHODS

Physa heterostropha, and probably other snails of the same genus, serve as the first intermediate hosts of *Posthodiplostomum minimum.* Twenty-three naturally infected snails were obtained from 2,351 snails collected from the pond in the New York Botanical Gardens. The snails were isolated in small vials in the laboratory with enough water to cover them. After forty-eight hours, the snails which did not shed cercariae were crushed to ascertain if they were infected. Naturally emerged cercariae and those from crushed snails were studied alive and in permanent mounts.

In the preparation of cercariae of *P. minimum* for study, it was found that hot water was the ideal killing agent. Complete eversion of the oral sucker was enhanced with this method. Conventional fixatives were not capable of causing this eversion, but were added after the sucker was protruded.

Daughter sporocysts were studied both alive and in sections. They were so tortuously threaded through the substance of the liver of the snails that it was impossible to separate a single one in its entirety; thus, portions had to be studied.

Seventy-five specimens of *Lepomis gibbosus* and *Lepomis megalotus* were seined from the pond from which the snails were collected and all were infected with the metacercaria of *P. minimum.* The sunfish were kept in the laboratory in a large concrete aquarium. Thirty-eight specimens of *Lepomis gibbosus,* sixteen of *Lepomis megalotus* and two of the minnow *Notemigonus chrysoleucas,* for use in infection experiments, were obtained by Mr. W. Bacha, of this department, from an artificial lake at Kent Cliffs, New York. These experimental fish were maintained in another large concrete aquarium. Ten specimens of *L. gibbosus,* five of *L. megalotus* and one of *N. chrysoleucas,* were examined for evidence of an infection with Neascus type metacercariae. All specimens were negative. Twenty-nine sunfish and the one specimen of *N. chrysoleucas* were used in infection experiments. The remaining ten fish served as controls and, on later dissection, all were negative.

The standard procedure used in all penetration experiments was to employ the total number of cercariae shed from two naturally infected snails over a twelve hour period. Experimental sunfish as well as the one minnow were exposed to cercariae for from one to five minutes, which was found sufficient for the penetration of large numbers without causing the death of the host. Four experimentally infected sunfish were sacrificed within five hours of penetration to determine the route or routes of migration of the cercariae to loci of development. Some of the remaining experimental sunfish were examined at intervals to study the development of the metacercariae. These larvae were compared in the living condition and in permanent mounts with specimens from the natural infections occurring in the sunfish from the New York Botanical Gardens.

The remaining laboratory infected sunfish and the naturally infected sunfish were used in feeding experiments with ducks, pigeons and one to four-day old chicks. Pigeons and ducks were fed the viscera of three infected sunfish. Chicks were fed the viscera of a sunfish containing 100 to 150 metacercariae before any other type of food was given.

The adults, which mature in about thirty-two hours, were obtained by stripping the mucosa of the small intestine. They were studied alive, in fixed and stained whole mounts, and in sections.

Eggs were recovered from the chick droppings after thirty-four hours. Hatching experiments were attempted but all were unsuccessful.

A single minnow, *N. chrysoleucas,* was exposed to cercariae four times for one-half hour with a period of ten days between exposures. Penetration of cercariae was seen on each occasion and the fish exhibited great discomfort.

All whole mounts were fixed in either Gilson's or Bouin's fixative and stained in Semichon's acetocarmine, paracarmine, Ehrlich's or Delafield's hematoxylin. Sections were cut in Tissuemat at seven to eight microns and stained with Mallory's triple stain or Heidenhain's hematoxylin.

Living material was studied by phase and ordinary light microscopy, unstained and with the use of intra vitam dyes, neutral red and nile blue sulphate.

The type and six paratypes of *Posthodiplostomum minimum,* deposited in the United States National Museum, were obtained through the courtesy of Dr. E. W. Price. All slides of *P. minimum* (No. 35096) were so opaque that the internal structure was indiscernible. Permission was granted by Dr. Price to demount and restain one of the specimens. Accordingly, it was bleached by the chlorine method

of Mayer (Microtomist's Vade Mecum, Lee, Tenth Edition; p. 254 #611) and then placed in a 0.25% solution of tribasic sodium phosphate (commercial grade) to restore the highly contracted cuticle of the hind-body to a more natural shape. The specimen was then stained in paracarmine, cleared and mounted. All internal organs remained unchanged in shape and position during the treatment. The re-stained specimen proved satisfactory for morphological study and comparison with adults obtained from feeding naturally and experimentally infected sunfish to chicks. The type specimen of *Neodiplostomum orchilongum* Noble, 1936 (U.S.N.M. No. 8922). and alcoholic preparations (Nos. 4843 and 4844) of metacercariae encysted in the visceral organs of centrarchids, identified by Linton in 1891 as *Diplostomum cuticola,* were also studied.

H. M. Miller's original slides of *Cercaria multicellulata* were made available for study through the courtesy of Dr. H. W. Stunkard. The preparations, however, proved unsatisfactory for the study of the finer morphological details of the cercaria.

Specimens of metacercariae and adults identified as *P. minimum* from the personal collections of Dr. M. S. Ferguson were generously loaned for study.

RESULTS

The eggs of *Posthodiplostomum minimum* are unembryonated when passed with the feces of the definitive host. Eggs recovered from feces were washed and placed in tap, boiled tap and pond water, but development never proceeded beyond the twelve to fourteen cell stage. Miracidia and mother sporocysts were not available for study because of the failure to hatch the eggs of *P. minimum.*

Stages identified as daughter sporocysts were obtained from naturally infected snails. They were found threaded through the tissue of the liver. Their free anterior ends moved about slowly and cercariae emerged from a narrow birth pore located sub-terminally. Gross dissections of two infected snails and serial sections of two others showed that the mantle cavity contained many free cercariae which escape to the exterior through the respiratory pore.

It was found, over a two year period, that the percentage of infection of *Physa heterostropha* in the early spring was small (0.2 per cent). Toward the end of July, the percentage of infection began to increase, due to the infection of juvenile snails, and reached a maximum of 8 per cent in September.

The cercariae, when undisturbed, were rather uniformly distributed throughout the water. They were suspended with the body straight, pointed downward and the tail-stem held upright with the furcae spread at an angle of 120° to 130°. The cercariae slowly sank but regained position by swimming upward, tail foremost, in rapid dashes. Many individuals, when they sank to the bottom of a container, adhered with the oral sucker and slowly moved about by gentle tail movement before resuming the suspended position.

The greatest numbers of cercariae escaped from the snails during the late afternoon. A light placed above an infected snail encouraged emergence and the cercariae, being positively phototrophic, were attracted to light areas in the container.

Penetration of the sunfish *Lepomis gibbosus* and *Lepomis megalotus* by naturally emerged cercariae of *P. minimum* was observed in the laboratory. When cercariae touched a fish host they adhered with the oral suckers. By gentle lashing

of the tail a cercaria moved to an area between the margins of the scales where penetration began. The tail lashed vigorously in this process and the oral sucker was continually inverted and everted. As soon as the first breach had been made in the host's tissue, peristaltic contractions which proceeded posteriorly and alternate contraction and extension in length aided the cercaria in its entrance. The tail was shed shortly after penetration had begun; therefore, it would seem that its usefulness is only in the initial breaching process. The entire body of the cercaria was within the host's tissue in from five to six minutes. Extreme discomfort was experienced by the sunfish during the penetration process. Greater numbers of cercariae penetrated and their actions seemed to be more vigorous when the water temperature was raised from 20° to 25° C.

Davis (1936), studying penetration by the cercariae of *Diplostomum flexicaudum* into different species of tadpoles, observed the entrance of the cercariae into veins and arteries. He concluded that the important route of migration for this species was via the blood stream.

Four experimental sunfish were dissected within a five hour period after exposure to cercariae of *P. minimum* in order to determine whether the migration in this host is an active penetration through the tissues or a passive distribution by the circulatory system to final loci of development. In experimental infections, large numbers of metacercariae were found in the general systemic circulation within one hour and in the hepatic sinuses and kidneys within three hours. At five hours, a few had penetrated through the somatic musculature and were free in the coelom or just exterior to it. The comparatively short time within which large numbers of metacercariae were found in the hepatic sinuses and in the kidneys indicates their passive distribution by both the hepatic and renal portal systems as well as by the general systemic circulation. Metacercariae may enter visceral organs directly from the vascular system or by penetration of the somatic musculature. The writer's observations demonstrate that the blood stream is an important route of migration for cercaria *P. minimum*.

During the first ten days after exposure, there was a reduction in the number of metacercariae found in the liver. Concomitantly, there was a three-fold increase in the size of the metacercariae. The numerical decrease of metacercariae resulted from an active migration of some of the metacercariae to organs in close proximity to the liver. Metacercariae migrated to the periphery of the liver where some emerged and attached to the surface of the stomach, small intestine and spleen. The penetration glands persisted until the fifteenth to twentieth day; therefore, it is assumed that the glands were still active during this migration. It is probable that the three-fold increase in size of the metacercariae in the liver results in "crowding" and numbers of metacercariae leave this organ to encyst in or on neighboring structures.

After a membranous cyst is formed by the larva, the host may deposit a thin fibrous covering on it in about thirty days. At this time the larvae become infective for the final host. There was no fibrous coat on cysts in the liver or kidneys. Maturity of metacercariae was determined by feeding cysts to day-old chicks and recovering adult worms within thirty-two hours. The metacercariae from experimental infections were identical with those derived from natural infections in sunfish. The adults which developed from both were identical. The adult worms con-

tained small numbers of eggs after thirty-four hours in the chick host and began to shed them soon afterward. The maximum number of eggs in a single adult was eight. The number of adults recovered after feeding 100 to 150 metacercariae ranged from 62 to 75.

The site of the infection was in the first four inches of the small intestine where *P. minimum* was found securely anchored to the mucosa by its oral sucker and holdfast organ. The hind-body was held at a right angle to the fore-body with the bursa copulatrix everted and directed toward the lumen between the free ends of the villi. This position of the hind-body facilitated copulation with neighboring individuals which was observed occasionally.

Small numbers of eggs were shed from the adults over a five to six day period. After that time, egg production decreased rapidly and the number of worms was gradually reduced. Two weeks after the development of adults, chicks retained only three to eleven strigeids. *P. minimum* adults were not recovered from the droppings; therefore, it is believed that they probably succumb and are digested in the host's intestine.

Pigeons and ducks used in the feeding experiments were refractory to infection at all times. Chicks which were older than four days or which had been fed before giving them metacercariae, were refractory to infection.

The single minnow, *N. chrysoleucas,* which was exposed to cercariae of *P. minimum* over a forty day period was sacrificed at eighty-one days. The minnow was negative for a metacercarial infection with *P. minimum.*

The metacercariae obtained in experimental and natural infections were compared and found morphologically identical with specimens (Nos. 4843 and 4844 U.S.N.M.) which were identified by Linton in 1891 as *Diplostomum cuticola.* The adults obtained in the present investigation were identical with *P. minimum* (Mac Callum, 1921) (No. 35096 U.S.B.A.I.) and *Neodiplostomum orchilongum* Noble, 1936 (No. 8922 U.S.N.M.).

The adult specimens from Ferguson's collection obtained from naturally infected *Ardea herodias* and *Ardea egretta* (American egret) and from chicks after feeding metacercariae from *Hyborhynchus notatus, Helioperca macrochira* and *Lepomis megalotus* were morphologically identical with the type specimens and adult *P. minimum* obtained in this study. There was some variation in size, however, which seemed to be dependent upon the metacercarial host. Adults obtained from metacercariae infecting *H. macrochira* and *L. megalotus* were larger in general than those from *H. notatus.* However this size difference was not a specific character as overlapping did occur. It was observed also that "situs perversus" of the ovary and anterior testis occurs much more frequently in Ferguson's collections than in adults obtained in the present investigation.

The metacercariae from minnows and sunfish in Ferguson's collection were identical with the metacercariae obtained by the author from sunfish. They were identified as *Neascus van cleavei.*

The egg: The egg of *Posthodiplostomum minimum* measures 0.080–0.091 mm. long (average 0.085 mm.) and 0.062–0.071 mm. broad (average 0.065 mm.). The egg is oval with the opercular more convex than the adopercular end. The shell is yellowish and moderately heavy. The ovum lies near the opercular end and is surrounded by vitelline cells and fluid.

The daughter sporocyst: Entire daughter sporocysts could not be dissected from the snail liver. Posterior to the sub-terminal birth pore, the sporocyst wall contains a considerable amount of yellowish pigment. The cercariae develop in chambers, one to about twenty in each, as broad as 0.250 mm.; constrictions between chambers are 0.004 mm. to 0.050 mm. in diameter; the lumen continuous throughout. Masses of germinal cells were seen in certain of the chambers of the sporocyst. The longest portion of sporocyst dissected from the snail liver measured 9 mm.

The cercaria: The cercaria of *P. minimum* (Fig. 1) is of the longifurcate monostome type. An acetabulum is not present although a group of small cells, the anlage of such an organ, is present. Although a fully-formed pharynx was not present, occasionally there was an aggregation of cells indicative of such a structure in a small area posterior to the oral sucker. No esophagus or ceca were observed. The oral sucker is oval, elongate and rather weakly developed. The anterior portion of this organ is constantly inverted and everted when the body of the cercaria is in contact with a surface, but no movement is seen when the cercaria is suspended in water. It occupies the anterior one-third of the body.

The body of the cercaria exhibits six annulations beginning at the posterior third of the oral sucker and usually extending to the level of the first pair of penetration glands. These annulations are very pronounced when the cercaria is suspended in water or lashing its tail. When placed under a coverslip, the crenations are not observable unless the cercaria is active. The crenations are probably caused by the contraction of circular muscle bands.

In the extended position, small posteriorly directed spines cover the anterior surface of the cercaria for one-third the length of the oral sucker, except at the extreme anterior end where there is a circum-oral spineless area. Spines, posterior to the crown of spines about the oral sucker, are confined to ten single rows. The first six rows are associated with body annulations. The terminal row of spines is at the level of the last pair of penetration glands. Each row is composed of spines as small as, or smaller than, those composing the anterior crown. A pair of long, flexible, posteriorly directed setae emerges laterally from the body at the level of the most posterior pair of penetration glands. These setae are longer than others present on the tail-stem.

There are eight pairs of flame cells in the body of the cercaria plus two in the tail-stem. Two pairs are located in the anterior half of the body. The other six pairs are in the posterior half. The excretory ducts could not be followed in their entirety, due to the very dense parenchyma. Immature cercariae from crushed snails also contained a very dense parenchyma which obscured the excretory vessels. A bipartite excretory bladder, containing an island of Cort, occupies the most posterior portion of the body. Collecting ducts leave the anterio-lateral edges of the bladder and are lost laterally in the dense body parenchyma.

A pair of simple eye spots, composed of a varying number of irregularly shaped dark brown pigment granules, is located at about two-thirds of the length of the body from the anterior end.

There are three pairs of penetration gland cells in the posterior third of the body. The first pair is separated from the posterior pairs by a compact mass of small cells representing the anlage of the acetabulum. Ducts lead anteriorly from the penetra-

tion gland cells through the oral sucker and open at small pores which are in two groups of three at each lateral margin of the circum-oral spineless area. Fluid secretions from the pores were evident under slight coverslip pressure. Secretory droplets remained at the openings of the pores due to surface tension which is strong enough to prevent their breakdown. The pair of ducts from the anterior glands are ventral to those from the two other pairs. Posterior to the last pair of penetration gland cells, there is a compact mass of small cells, which is the anlage of the genital complex.

The tail of the cercaria is longer and slightly narrower than the body. Five pairs of caudal bodies are contained in the tail-stem. There is some difference in the refractive indices of these bodies even among cercariae from the same snail. In certain cercariae their borders are clear; in others vague; and in some they cannot be detected at all. The caudal bodies are most readily seen in moribund cercariae. There is some shifting in position of the posterior pairs when the cercaria is very active, but they never reach the furcae. In the spaces between the caudal bodies there are two pairs of flame cells, one pair on each side of an excretory duct that courses from the posterior portion of the excretory bladder through the center of the tail-stem. The duct bifurcates at the level of the furcae and each duct proceeds to the proximal anterior surface of a furca where it empties by a minute pore. The surface of the tail-stem is very finely annulated and bears sensory setae in three groups; two anterior groups immediately posterior to the body and a posterior group immediately anterior to the furcae. The three distinct groups of setae are always maintained, but the numbers of setae in each vary. Very fine and short setae are present on the anterior surface of the furcae. It was only possible to see these structures with the phase microscope. The distal anterior half of each furca bears a thin fin which is continuous with one of the same length from the posterior margin.

Measurements of twenty-five suecimens fixed in hot 5 percent formalin are: body length 0.192 mm. (0.112–0.226), body width 0.032 mm. (0.020–0.046), tail stem length 0.247 mm. (0.171–0.254), tail-stem width 0.028 mm. (0.019–0.038), furcal length 0.219 mm. (0.175–0.226).

The metacercaria: Development: (all measurements are averages).

Five days—0.195 mm. × 0.034 mm. There was little increase in size over that of the cercaria and no regression of structures was visible.

Ten days—0.314 mm. × 0.092 mm. The metacercaria had increased considerably in size and very narrow ceca were observed in the area of the penetration gland ducts. The complicated reserve bladder began to form at this time

Fifteen days—0.901 mm. × 0.264 mm. There was a great increase in size, a pharynx was clearly seen, but the acetabulum, hold-fast organ and genital primordia showed little development. The penetration glands and ducts began to show signs of regression.

Twenty days—0.940 mm. × 0.290 mm. There was little increase in size. Penetration glands and ducts had almost completely regressed, exposing the pharynx and esophagus. The genital primordia were visible as two distinct groups of cells and the reserve bladder was complete. The acetabulum and hold-fast organ were well developed. The constriction separating fore- and hind-body was visible. The membranous cyst, a product of the parasite's secretion, closely invested the metacercaria.

Twenty-five days—1.23 to 1.36 mm. × 0.640 to 0.680 mm. The metacercaria had reached its maximum size. The shape resembled that of the adult except that the hind-body was globular instead of cylindrical. The genital complex was composed of five elements corresponding to the adult organs.

Thirty days—no increase in size. Metacercariae were fully developed and the membranous cyst was no longer closely applied to the parasite.

A description of the mature metacercaria is not included as it has been adequately described by Hughes (1928) based on forms obtained from *Lepomis gibbosus.* A description of the adult is also omitted since Noble (1936) and Dubois and Rausch (1948) have carefully described the form.

The writer, however, includes data on the spines of the adult cuticle not reported in previous descriptions. Small posteriorly directed spines are present over the ventral surface to a level 0.480 mm. from the anterior end. Concentric rings of spines are present also on the internal surface of the hold-fast organ and gradually disappear over the external surface. No other spines are evident on the body.

DISCUSSION

In the identification of strigeid cercariae, minor differences in the pattern of cuticular spines, the presence or absence of suckers, size and number of penetration glands, presence and number of caudal bodies and the arrangement of the excretory system serve as distinguishing specific characters.

The cercaria of *Posthodiplostomum minimum,* described in this report, is morphologically distinct from *Cercaria multicellulata,* which was reported by Ferguson (1937), and Hunter (1936, 1937) and Hunter and Hunter (1940) as a larval stage of *P. minimum.*

The cercaria of *P. minimum* can be distinguished from *C. multicellulata,* as described by H. M. Miller (1923), by its large size, the absence of anteriorly directed spines at the orifice of the oral sucker, the presence of body setae, the five pairs of caudal bodies, the smaller crown of spines over the anterior surface of the cercaria, the occurrence of tail-stem setae in three distinct groups and the continuous fins at the distal ends of the furcae. Miller did not describe spines on the body as occurring in rows, but did state, ". . . there is indication of sparse spination in somewhat definite bands back to the level of flame cell II." This would be approximately one-half the distance from the oral sucker to the first pair of penetration glands. Spines on the body of the cercaria of *P. minimum* terminate at a much more posterior level. The excretory pattern of the two cercariae is identical, except that in *P. minimum* the last pair of flame cells in the body has never been observed in the position described for *C. multicellulata.*

The experiment determination of *C. multicellulata* as a larval stage of *P. minimum* by Ferguson (1937) is doubtful. Examination of Ferguson's slides verifies the identify of all metacercariae as *Neascus van cleavei.* However, since minnows used in life history experiments were naturally infected before exposure to *C. multicellulata* and since this report demonstrates another morphologically distinct cercaria as the larval stage of *P. minimum,* the life history relationships of *C. multicellulata* are unknown.

Hunter (1936, 1937) and Hunter and Hunter (1940) obtained metacercariae identified as *N. van cleavei* developing in the sunfish, *L. gibbosus,* after exposure to

cercariae identified by them as *C. multicellulata.* However, no feeding experiments were reported with experimental metacercariae.

Brooks (1948) reported *Cercaria louisiana* from *Physa sayii* in Wisconsin. In this report, he illustrated morphological characters, such as rows of spines on the body, solid eye spots, and two pairs of flame cells in the tail stem, which were not included in the original description of the species by E. L. Miller (1935). E. L. Miller did not describe or illustrate the excretory system. In the original description, he stated that the penetration gland ducts, ". . . extend forward as bipartite structures. . . . The inner part of each duct connects with an outer gland, while the outer part divides into two ducts each connecting with one of the two posterior glands." His contention that the penetration gland ducts are bipartite structures is not compatible with present knowledge of cercarial morphology, according to which each gland has its own distinct duct. Because of this erroneous interpretation and the inadequacy of the description which was devoid of characteristics for distinguishing the form from all other furcocercous monostome cercariae, according to Article 25 of the Rules of Zoological Nomenclature as revised by the 11th International Congress, *Cercaria louisiana* is an invalid name and its use by Brooks (1948) is considered a misnomer.

In the same report, Brooks suppressed *C. louisiana* as a synonym of cercaria *P. minimum.* His figure of *C. louisiana* is similar to the form described in this report and the measurements fall within the latter's range; the pattern of body spines is identical, as is the continuity of the fin folds. Brooks, however, did not describe caudal bodies, setae on the tail-stem or body and illustrated the excretory ducts as opening at the distal ends of the furcae.

In the same report, Brooks stated that for three years he had not recognized that the larvae from *P. sayii,* identified as *C. louisiana,* actually consisted of two species, the second of which he described as a longifurcate apharyngeal monostome cercaria, *"Cercaria Zeta."* He distinguished it from the cercaria, which he had identified as *C. louisiana,* by the presence of five pairs of caudal bodies, "flagellets" (setae?) on the tail stem and a pair on the body, as well as other morphological differences and also by its inability to penetrate sunfish. Therefore, it can be concluded that Brooks did not overlook setae on *C. louisiana* as their absence was used as a character for the differentiation of *"C. Zeta." "C. Zeta,"* although similar to the cercaria used in the present investigation, can be differentiated by such morphological characters as the pattern of the body spines and the position of the genital anlage in relation to the penetration glands, the excretory pattern, and its inability to penetrate sunfish.

From the preceding discussion, it is apparent that cercaria *P. minimum,* used in present research, can be distinguished from cercariae previously postulated as larvae of *P. minimum.*

Klak (1939) found members of the CYPRINIDAE, with the exception of *Notemigonus chrysoleucas,* infected with *Neascus van cleavei* in a pond which also contained uninfected members of the CENTRARCHIDAE. The multiple exposure of one specimen of *N. chrysoleucas* to cercariae of *Posthodiplostomum minimum* by the writer, and the subsequent failure of the cercariae to develop in this host verifies Klak's observation in regard to this species.

It is the writer's opinion that Ferguson's (1943) report of the infection of trematode-free herons with metacercariae from both fish families indicates a possible

confusion of two closely related strigeids as *P. minimum*. Metacercariae from centrarchid fishes were fed to one group of herons, the other group receiving metacercariae from cyprinid fishes. Miracida from the eggs of adult strigeids which reached maturity in the herons infected both *Physa acuta* and *Physa heterostropha*. The cercariae produced from the centrarchid line of metcercariae infected only fish of the CENTRARCHIDAE while those of the cyprinid line infected only the CYPRINIDAE. The metacercariae in both cases were similar. A critical morphological study of the cercariae from the two lines was not undertaken, nor was a study of the adults reported. Examination of the controls showed that all fish used in the experiments harbored natural infections with Neascus type metacercariae. The natural infection of experimental fish as well as the lack of a critical morphological study makes it uncertain whether Ferguson was actually dealing with a single species or two closely related species.

Hoffman's (1950) report that *Cercaria louisiana* of Brooks develops to *Neascus van cleavei* only in the CENTRARCHIDAE, seems to indicate that there are at least two species whose metacercarial stages are morphologically similar but which can be distinguished in the cercarial stage.

The confusion which exists regarding cercariae and metacercariae thought to be larval stages of *P. minimum* has arisen through the attempt of investigators to relate cercariae to adults on the basis of similarities between experimentally produced metacercariae and those found in nature, which developed to *P. minimum in* experimental hosts. The elimination of this confusion necessitates carefully controlled life-history studies from cercaria to adult with the larval stages hitherto thought to be *P. minimum*.

SUMMARY

1. The first carefully controlled experimental life history of *Posthodiplostomum minimum* from cercaria to adult is reported.

2. *Physa heterostropha* may serve as the first intermediate host.

3. Evidence is given for the passive transfer of metacercariae via the circulatory system to loci of development in *Lepomis gibbosus* and *Lepomis megalotus*. A description of this development is given.

4. Adults are obtained in thirty-two hours after feeding metacercariae to young chicks. Eggs shed by these worms did not undergo development.

5. The cercaria is morphologically distinct from *Cercaria multicellulata* H. M. Miller, 1923 and *Cercaria louisiana* E. L. Miller, 1935, each of which was previously reported as the cercarial stage of *P. minimum*.

6. The metacercariae are identical with *Neascus van cleavei* (Agersborg, 1926). The adults developing in chicks from these metacercariae are identical morphologically with the type specimens and descriptions of *P. minimum* (Mac Callum, 1921) Dubois, 1936.

REFERENCES

AGERSBORG, H. P. 1926 Studies on the effect of parasitism upon the tissues. II. Arch. Schiffs- und Tropen-Hygiene, **30**: 18–30.

BROOKS, E. G. 1948 Report to the Biology Division of the Wisconsin Conservation Department and the Department of Zoology, University of Wisconsin, on larval trematode parasites of Carrol Lake snails. (in press.)

DAVIS, D. J. 1936 Pathological studies on the penetration of the cercaria of the strigeid trematode, *Diplostomum flexicaudum*. J. Parasit. **22**: 329–337.

DUBOIS, G. 1936 Nouveaux principes de classification des trématodes du groupe des Strigeida. Rev. Suisse Zool. **43**: 507–515.
———1937 Sur quelques Strigéidés. Rev. Suisse Zool. **44**: 391–396.
———1938 Monographie des Strigeida. Mém. Soc. Neuch. Sci. Nat. **6**: 535 pp.
——— AND RAUSCH, R. Seconde contribution à l'étude des Strigéidés (Trematoda) Nord-Américains. Bull. Soc. Neuch. Sci. Nat. **71**: 29–61.
FERGUSON, M. S. 1936 Experimental studies on *Neascus van cleavei*. J. Parasit. **22**: 544–545.
———1937 Experimental studies on *Posthodiplostomum minimum* (Mac Callum, 1921), a trematode from herons. An abstract of a thesis, University of Illinois, Urbana, Illinois.
———1938 Experimental studies on *Posthodiplostomum minimum* (Mac Callum, 1921), a trematode from herons. J. Parasit. **24** (Suppl.) : 31.
———1940 Excystment and sterilization of metacercariae of the avian trematode *P. minimum* and their development into adult worms in sterile cultures. J. Parasit. **26**: 359–372.
———1943 Experimental studies of the fish hosts of *Posthodiplostomum minimum*. J. Parasit. **29**: 350–353.
HOFFMAN, G. L. 1950 Studies on the development and Biology of two trematodes, *Bunodera eucaliae* and *Posthodiplostomum minimum*, Thesis, Department of Zoology, Graduate College of the State University of Iowa, Iowa City, Iowa.
HUGHES, R. C. 1927 Studies on the trematode family Strigeidae (Holostomidae) No. VI. Trans. Amer. Micros. Soc. **46**: 248–267.
———1928 Studies on the trematode family Strigeidae (Holostomidae) No. IX. Trans. Amer. Micros. Soc. **47**: 320–341.
———1929 Studies on the trematode family Strigeidae (Holostomidae) No. XIV. Occasional Papers of the Mus. of Zool., Univ. of Mich., No. **202**: 1–29.
HUNTER, G. W. 1936 Penetration of the common sunfish by the holostome cercaria, *C. multicellulata*. J. Parasit. **22**: 542.
———1937 Parasitism of the fishes in the lower Hudson area. Suppl. 26th Ann. Rep. N. Y. St. Consv. Dept., 1936, **11**: 265–273.
——— AND HUNTER, W. S. 1935 Further studies on fish and bird parasites. Suppl. 24th Ann. Rep. N. Y. St. Consv. Dept (1934) **9**: 267–283.
——— AND ———1940 Studies on the development of the metacercaria and the nature of the cyst of *P. minimum*. Trans. Amer. Micros. Soc. **59**: 52–63.
KLAK, G. E. 1939 Neascus infestation of black-head, blunt-nosed and other forage minnows. Trans. Amer. Fish Soc. **69**: 273–278.
LEIDY, J. 1856 A synopsis of Entozoa and some of their ectocongeners observed by the author. Proc. Acad. Nat. Sc., Phila. **8**: 42–58.
LEUCKART, R. 1889 Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. 2. Aufl., 1, 4. Lief., 2. Abt: 97–440.
LUTZ, A. 1921 Zur Kenntnis des Entwicklungszyklus der Holostomiden. Centralbl. Bakteriol., Abt. 1, Orig. **86**: 124–129.
MAC CALLUM, G. A. 1921 Studies in helminthology. Zoopathologica, N. Y. Zool. Soc. **1**: 135–204.
MATHIAS, P. 1922 Cycle évolutif d'un trématode holostomide (*Strigea tarda* Steenst.). C. R. Acad. Sc., Paris, **175**: 599–602.
MILLER, E. L. 1935 Studies on North American cercariae. J. Parasit. **21**: 244–254.
———1936 Studies on North American cercariae. Illinois Biol. Monogr. **14** (2) : 1–125.
MILLER, H. M. 1923 Notes on some furcocercous larval trematodes. J. Parasit. **10**: 35–46.
———1925 Comparative studies on furcocercous cercariae. Illinois Biol. Monogr. **10**: 1–112.
NOBLE, A. E. 1936 New avian trematodes of the genus *Neodiplostomum*. J. Parasit. **22**: 247–254.
RUSZKOWSKI, J. 1922 Die postembryonale Entwicklung von *Hemistomum alatum* Dies. auf Grund experimenteller Untersuchungen. Bull. Int. de l'Acad. Polon. Sci. Lettres, Cl. Sci. Math. Nat., Ser. B, Sci. Nat. (1921) : 237–250.
VAN CLEAVE, H. J. AND MUELLER, J. F. 1934 Parasites of Oneida Lake fishes, Part III. Roosevelt Wild Life Ann. **3**: 155–334.

Explanation of Plate

Fig. 1. Ventral view of cercaria *Posthodiplostomum minimum.* Composite drawing made from living specimens with the aid of a camera lucida. (×250)

Fig. 2. Ventral view of *Cercaria multicellulata* after H. M. Miller. (×257)

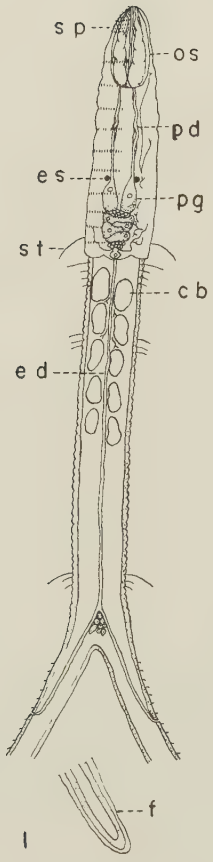
Fig. 3. *Cercaria louisiana* after Brooks. No scale given by Brooks.

Fig. 4. *Cercaria louisiana,* dorsal view, after E. L. Miller. Scale represents 0.625 mm.

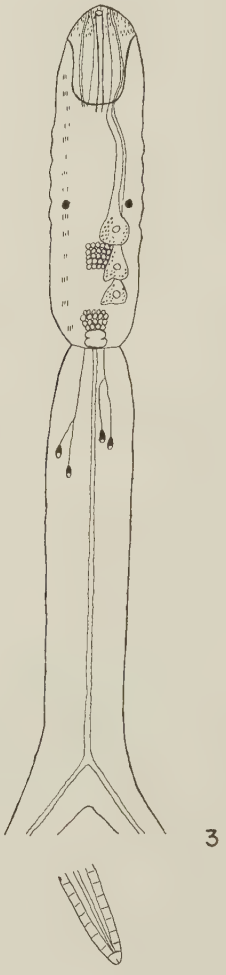
Fig. 5. *Cercaria louisiana,* dorsal view, after E. L. Miller. Scale represents 0.05 mm.

Abbreviations used: cb = caudal body; ed = excretory duct; es = eye spot; os = oral sucker; pd = penetration gland duct; pg = penetration gland; sp = spines; st = seta; f = fin.

PLATE I

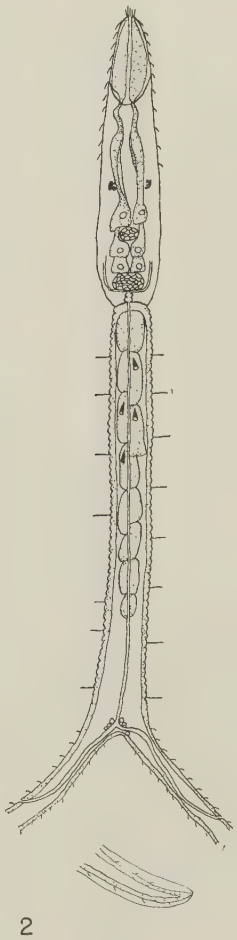


1

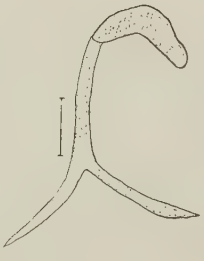


3

4



2



5

TREMATODE, GREGARINE AND FUNGUS PARASITES OF *ANOPHELES* MOSQUITOES

PIETER HENDRIK VAN THIEL

Laboratory of Parasitology, University of Leyden, and Parasitological Department, Leyden Institute of Tropical Medicine

Each research worker in the field of malariology, when dissecting *Anopheles* mosquitoes, comes across many parasites which have been described in a great number of papers. Christophers (1952) has recently summarized the recorded parasites of mosquitoes.

Insufficient justice has been done to former investigations on trematodes in adults and larvae of *Anopheles* species and on their life history in this paper by Christophers and in papers by Jones (1950, more particularly dealing with *Anopheles* larvae as intermediate hosts for larval trematodes) and by Walandouw (1934, describing mainly nematode parasites of *Anopheles* larvae).

For this reason a short addition is given concerning trematodes in *Anopheles*, and a few new observations on gregarines and fungus in *Anopheles* mosquitoes are described under B and C.

A. TREMATODES

Christophers (1952) only mentioned that encysted trematodes are not uncommon in *Anopheles* larvae and adults. Walandouw (1934) stated that the life cycle of the trematodes, found by Grassi in anophelines, is unknown. Jones (1950), while carrying out a survey in Ceylon, found a large number of larvae of *A. culicifacies* Giles, *aconitus* Dön. and *annularis* Wulp infected with cercariae, called by him *Cercaria anophelini*, but no cysts were evident. The author believed that encystment occurs in the adult mosquito, probably during the short pupation period. He was unable to carry out further work on the life history of this trematode, but expected to find the adult fluke in a winged animal, either the mammalian bat or a bird, possibly of the swallow family.

In papers published from 1921 to 1930 the present author, however, elucidated this life history. Five per cent of female *A. maculipennis* Mg. caught in Leyden (Netherlands) were found infected with encysted metacercariae, called *Agamodistomum anophelis* (van Thiel, 1921). In a further paper (van Thiel, 1922) details of the structure of this parasite were given. It was also found in *Anopheles* larvae and in adults bred from larvae and pupae caught in ditches. We succeeded in discovering in these ditches the corresponding cercaria with the aid of the following characteristics: stylet (this is often found in the cysts in the *Anopheles* larvae), size of the suckers and Y-shaped excretory bladder. This cercaria, called *Cercaria anophelis*, belongs to the *Cercariae ornatae* of Lühe, and develops in a sporocyst found in the liver of *Planorbis vortex* L. It has been proved that these cercariae may infect laboratory reared *Anopheles* larvae where they encyst. Cercariae and metacercariae were figured. The paper concluded with some remarks about the definitive host; the suppositions expressed earlier were stated to be very improbable.

Received for publication, August 5, 1953.

Alessandrini (1909) presumed the adult fluke to be *Lecithodendrium ascidia* (v. Ben.) of bats. I examined three bats (*Vesperugo pipistrellus* Schreb.) caught in Leyden and found this fluke in their intestines. It showed, however, a V-shaped excretory bladder. Von Linstow (1888) associated with this parasite another metacercaria, found by him in larvae of *Ephemera, Perla* and *Chironomus plumosus* L. Von Linstow was probably correct, because we succeeded in finding encysted metacercariae with a horseshoe-shaped excretory bladder in *Chironomus*.

Alessandrini's presumption that the adult of the encysted metacercariae found by Ruge and von Linstow would be *Sphaerostomum bramae* O. F. Müll., is incorrect. We found the latter species near Leyden in the fish *Leuciscus rutilus* L. Its cercaria is *Cercaria micrura* Fil., whose excretory bladder and the structure of its intestinal tract and suckers resemble in every respect those of *Sphaerostomum*.

Brandes, quoted by Ruge (1903), was of the opinion that the adult fluke lives in a bird. Of the birds which occurred near the breeding places of infected anophelines in Leyden, only the swallow (*Hirundo rustica* L.) could be considered. We found numerous specimens of *Plagiorchis maculosus* Rud. with a distinct Y-shaped excretory bladder in the intestines, however with a very short esophagus. Lühe (1909) very probably rightly declared the metacercariae found by von Linstow (1897) in *Notidobia ciliaris* L. and *Sialis lutaria* L. to be larval forms of this fluke. *Cercaria secunda,* described by Ssinitzin (1905), is probably the cercaria of a species of *Plagiorchis*.

In bats a species of *Plagiorchis* also occurs, namely *P. vespertilionis* O. F. Müll. We have been able to examine many specimens of this fluke. The absence of the esophagus, the presence of broad intestinal branches and the fact that van Beneden (1873) described young specimens having the typical structure of the intestines of *Plagiorchis,* made me conclude that *Agamodistomum anophelis* does not belong to a species of *Plagiorchis*.

It remained for me to examine whether Soparkar (1917) rightly presumed that the adult fluke of the metacercariae found by him in Bombay (called by us *Agamodistomum sintoni*) should be assigned to *Clinostomum* Leidy of aquatic birds, mainly *Ardeidae,* which eat fish. Near Leyden, at the place where we made our investigations, only the barn-swallow could be considered. No species of *Clinostomum* has been found. Moreover, because of the morphology of *Clinostomum, Agamodistomum anophelis* is not its larval stage. Braun (1879–1893) and Lühe (1909) presumed that the infection of *Ardeidae* in Europe with *C. heterostomum* Rud. and *complanatum* Rud. takes place through the intermediary of infected fishes in the Mediterranean territory. Ciurea (1911) has found *C. complanatum* encapsulated in *Perca fluviatilis* L., from the Lower Danube. Even if the development of *Agamodistomum sintoni* was correctly observed by Soparkar, this development does not apply for the agamodistomum found by me.

Finally van Thiel (1930) proved that the metacercariae found in *A. maculipennis* near Leyden develop into *Pneumonoeces variegatus* Rud. in the lungs of the frog *Rana esculenta* L. Seven very young trematodes were found in the lungs, moving in their typical manner 24 hours after the feeding of the frogs, kept in the laboratory in tap water without the presence of plants and insects and only artificially fed with earth worms.

The dimensions of the bodies and of the oral and ventral suckers of these flukes (in our 1922 paper the dimensions of oral and ventral suckers were wrongly exchanged) agreed with those of the metacercariae encountered in the *Anopheles* larvae. The typical granular contents of the excretory bladder, which was still Y-shaped, had only partly disappeared. One young fluke was found in the esophagus near the pharynx, so that it is nearly certain that the strongly mobile metacercariae made their way, after their release from the cysts in the intestinal tract, to the pharynx and afterwards through the glottis to the lungs. Frogs caught in ditches and meadows, where the infected *Anopheles* larvae were found, indeed, showed *Pneumonoeces* in the lungs.

The presence of *Pneumonoeces* eggs could not be demonstrated in the mucus secretion of the mouth of the frog, nor in its feces. The eggs resemble the eggs of *Prosotocus confusus* Lss. which was regularly found in the intestines of frogs.

Since it was impossible to perform the experiments with frogs reared in the laboratory, we were obliged to work with frogs caught in the meadows, after having established that these frogs did not harbor trematodes in the lungs. Although not probable, it was therefore not excluded that a few lung flukes would occur.

TABLE 1.—*Development and dimension of flukes after feeding of frogs with* Anopheles *larvae infected with* Agamodistomum anophelis

Frog	Time of examination after feeding	Number of flukes	Dimension
A	24 hours	1 in esophagus near pharynx, 7 in lungs	200 micra
B	9 days	7 in lungs	201–288 micra
C	1 month, 15 days	8 in lungs	0.56–2.62 mm.
D	4 months, 14 days	27 in lungs, a few mature	1.68–4.45 mm.
E	8 months, 13 days	8 in lungs, a few larval	2.4–4.88 mm.
F	1 year, 15 days	2 mature specimens in lungs	half the dimension of adults

In order to give more conclusive force to our experiments, a frog, caught in Aalsmeer, was kept in an aquarium for 4 months and fed only with earth worms. It was fed then with *Anopheles* larvae infected with metacercariae and killed after three days. We found very small free metacercariae wholly resembling those found in the cysts. At the same time with this frog, the above mentioned frog F (*v.* table) was fed with similar positive material. When examined after three days, two very young free metacercariae were found near the above mentioned mature flukes.

All reared flukes could be identified with the aid of the thesis by Wundsch (1911) as *Pneumonoeces variegatus* Rud. The species *P. asper* Lss. and *schulzei* Wdsch. have not been found. One specimen of *P. similis* Lss. has been encountered in the lungs of one frog caught near Leyden. According to Ssinitzin (1905) its metacercaria is found in *Calopteryx virgo* L. This may be doubted. Since encysted metacercariae from *Anopheles* sometimes show a slightly spined cuticula, it is not excluded that the development of the metacercaria of *P. similis* also takes place in *Anopheles*.

B. GREGARINES

Dr. D. Metselaar found in Dutch New Guinea a specimen of *A. farauti* Lav., which showed on the wall of the stomach two gametocysts of a gregarine, measuring 99×99 and 86×50 micra. They contained numerous round to angular zygotes, each originated by the conjugation of two probable isogametes, whose nuclei have

nowhere fused. In one of the two gametocysts a distinct residual protoplasm (arrow in fig. 1) was visible, quite resembling this body figured e.g. by Wenyon (1911) in *Lankesteria culicis* (Ross 1898).

The parasite probably should be placed in the order *Eugregarinida,* suborder *Haplocyta* (Lank.), family *Diplocystidae* Bhatia, 1930, according to the division described by Bhatia (1924, 1930).

The parasite should probably not be placed in the order *Schizogregarinida* (*Caulleryella anophelis* Hesse is a species of this order) because 1) schizogony, just as in *C. pipientis* (described by Bresslau and Buschkiel, 1919), occurs in the cycle of these gregarines. If schizogony occurred in the gregarine found in New Guinea, then surely more gametocysts or other developmental stages of the parasite would have been found. 2) Species of *Caulleryella* are parasites of the lumen of the intestinal tract, situated between epithelium and peritrophic membrane. 3) The nuclei of the two gametes as a rule fuse following conjugation in the insect larva.

According to Bhatia (1930) the subdivision of both orders into families is based 1) on the form of the gametocysts and sporocysts, 2) the shape of the trophozoite, 3) the form and character of the epimerite when present, and 4) the habitat of the parasite. The differences in the sporocysts, however, are considered to be fundamental. Only the gametocysts and the habitat of the parasite described above being known, a certain diagnosis cannot be made. As to the localization, by far the largest number of *Haplocyta* are parasites of the coelom or of organs in direct connection therewith (Johnson, 1902).

The presumption is obvious that the parasite is a species of the genus *Diplocystis* Künstler 1887, because the parasites included in this genus are intestinal or coelomic parasites of insects. *Diplocystis* has been recorded from the outer wall of the intestines of a larva of *Culex,* collected in Corsica by Léger and Duboscq (1902). The parasite has, however, not been described. Johnson (1902) described *Diplocystis* as a new protozoan parasite of *Anopheles* on the outer wall of the midgut, recalling oöcysts of the malaria parasite, in about 8 per cent of the females of *A. maculipennis* Mg. in Boston. The cysts varied extremely in size (12 to 80 micra). The numerous specimens represented only one phase of development. Not a single sporulating specimen has been found. All were simple cells, enclosed in a thin structureless membrane, and without epimerites or appendages of any sort. No evidence of fusion of nuclei or even of cell bodies in conjugation could be observed. The actual fecundation obviously came later. The parasite which we found in *A. farauti* is therefore in a later developmental stage than that found by Johnson. Although the localization is the same, it is not probable that Johnson's parasite and ours are identical.

Künstler (1887) described the genus *Diplocystis* after the type species *Diplocystis schneideri* from *Periplaneta americana* L. Léger (1904) mentioned as a feature of *Diplocystis* that two monocystic individuals soon associate to form a couple which grows up to the moment of the sporulation. Cuénot (1901) likewise considered the association of two individuals the most important character of *Diplocystis.* He observed in *D. minor* and *major* from *Gryllus domesticus* L. a great number of zygotes, whose nuclei frequently did not fuse. Bhatia (1930) mentioned as a feature of *Diplocystis:* "spores spherical or oval; trophozoites fuse precociously to form spherical masses, coelomic. Spores spherical or oblong, with eight sporozoites."

Because Johnson and Metselaar did not observe further stages of development, it is therefore not yet possible to say with certainty whether they observed the genus *Diplocystis*.

We wonder also whether the parasite encountered by Metselaar is the closely related *Lankesteria* Mingazzini 1891. *Diplocystis* and *Lankesteria* are joined by Bhatia in the family *Diplocystidae* Bhatia, without indicating a characteristic of this family. No distinct morphological differential feature common to both genera is mentioned. *Lankesteria,* however, is an intestinal parasite of tunicates, insects, etc. Cysts and stages in spore formation have been found in *L. culicis* (Ross) by Ganapati and Tate (1949) in the malpighian tubules of the pupae of *Aedes geniculatus* (Olivier). When the imagines emerge from the pupae the malpighian tubules are packed with the ripe spores which escape through the alimentary canal. Neither the whole nor partial absence of binucleated zygotes in *L. culicis* described by Wenyon (1911), Ray (1933), Ganapati and Tate (1949) points to this diagnosis.

The joining of the genera *Diplocystis* and *Lankesteria* in one family by Bhatia has been questioned by Ray (1933). The position of *Lankesteria culicis* among the haplocyte gregarines is, according to him, no longer justified by the presence of a well-developed epimerite, a rudimentary protomerite and the capacity for a completely extracellular development. All these points favor its inclusion among the septate (Cephaline) gregarines.

However this may be, the true diagnosis can only be made after the absence of more or less well-developed epimerites has been observed in young trophozoites.

The gregarine found by Metselaar in *Anopheles farauti* is therefore provisionally identified as *Diplocystis metselaari* n. sp., next to the species of *Diplocystis* found by Johnson in *Anopheles maculipennis,* for which the name *Diplocystis johnsoni* n. sp. is proposed.

C. FUNGI

During the examination of ovaries of Indonesian anophelines for age determination (van Thiel, 1953), we found in Java two female *A. tesselatus* Theob., whose ovaries were completely filled with yellowish structures resembling nematode eggs (fig. 2). These took the place of the eggs, of which no trace could be observed. A complete sterilization had therefore taken place. When these ovaries were examined a few days after being isolated from the insect's body and placed in fixing solution, it could not be determined whether these structures also occurred elsewhere in the body. Dr. Christophers in Cambridge, to whom I showed a photograph of these structures, thought that they are very like the sporangia of the fungus *Coelomomyces*. He was quite right.

Coelomomyces stegomyiae was described by Keilin (1921) from *Aedes albopictus* Skuse, collected in the Federated Malay States. The mycelium forms a dense ramification round the midgut and ceca, but does not show any transverse septa. Terminal swellings of the mycelial branches, ending in the development of smooth walled sporangia, become separated from the mycelium and are found free within the insect's body cavity. The young sporangium is filled with very dense protoplasm. In a stage of further development the sporangia are filled with lozenge-shaped spores.

Iyengar (1935) found the entire haemocele of larvae of seven species of *Anopheles* in India parasitized by *Coelomomyces indiana* and *Coelomomyces anophelesica*.

The mycelium is always attached to the fat-body. The wall of the sporangia of both species shows a definite sculpturing produced by well-developed ribs. The mycelium is broader than in *Coelomomyces stegomyiae*. An effect of the infection is the suppression of the imaginal buds. It seems improbable therefore that an infected larva would complete its development into the adult mosquito, but in a few exceptional cases the infected larva pupated and the adult emerged.

Manalang (1930) was the very first to report these sporangia, which he considered to be coccidia, from the haemocele of adult mosquitoes in the Philippines, 1 to 2 per cent being infected. A single larva of *A. tesselatus* Theo. showed a similar condition in the thorax and the first abdominal segments. Feng (1933), in Woosung, found infections in both adults and larvae of *A. hyrcanus* var. *sinensis* Wied. and *Culex tritaeniorhynchus* Giles.

The first investigator to find sporangia in the ovaries was Gibbins (1932) in Uganda. In the ovaries of one specimen of *A. gambiae* Giles many objects resembling *Ascaris* ova were seen. Also very numerous ovoid bodies, light brown in color, very probably also *Coelomomyces* sporangia, were seen in the ovaries of *A. costalis* and *A. funestus*.

Walker (1938) discovered sporangia of four types in the adults of *A. gambiae*. Only types 1 and 2 have been encountered in wild-caught adults, types 3 and 4 regularly in larvae. The infection in females occurs most frequently in the ovaries and causes sterilization. General infections are always fatal. Light infections in the female were almost always limited to the ovaries.

The species of *Coelomomyces* found by us in Indonesia completely agrees with type 1 encountered by Walker in Africa in *A. gambiae*. His types 1 and 4 measure between 45 and 60 micra in length, and types 2 and 3 from 25 to 30 micra. The sporangia found by us measure between 55 and 63 micra in length, and between 33 and 42 micra in breadth, so that these dimensions agree with those of Walker's types 1 and 4. Only his type 1 and our type ought to be identified by the structure of the sporangia. Walker, however, has given a name only to the commonest of the four forms, i.e. type 3, namely *Coelomomyces africanus*. Since it was very probable that Walker observed more than one species of this genus, we propose to denominate his type 1 and the type encountered by us in *A. tesselatus: Coelomomyces walkeri n. sp.*

The species observed by Manalang (1930) in the Philippines very probably agrees with Walker's type 4, the sporangia measuring 44.8×29 micra and having the general appearance of small abnormal *Ascaris* eggs because of the double-walled shell with longitudinal ridges. For both types we propose the denomination *Coelomomyces ascariformis n. sp.* Walker's types 2 and 3 are thought to be identical.

The sporangial wall of *Coelomomyces walkeri,* found in *A. tesselatus,* consists of two distinct layers: an internal, thin layer and an external layer of a yellowish color, not showing the numerous apertures which were observed by Keilin (1921) in the above mentioned *Coelomomyces stegomyiae*. The sporangia are filled with very dense protoplasm, in which a large number of very small nuclei could be distinguished with oil immersion lens. No indication of the formation of spores existed, which was observed by Keilin in *C. stegomyiae*.

In both females of *A. tesselatus,* mentioned above, egg-development was wholly inhibited, in agreement with the observations by Walker.

It could not be observed whether a mycelium was present in the haemocele, because the ovaries had been isolated from the abdomen. Only two hyphae measuring from 1.4 to 12.6 micra in thickness protruded from one of the ovaries; they appeared to penetrate from the haemocele into the interior of the ovary, after having surrounded the ovary on the spot. The ovaries were elsewhere surrounded by a mycelium only in a few spots. This mycelium is a thin-walled tubular structure

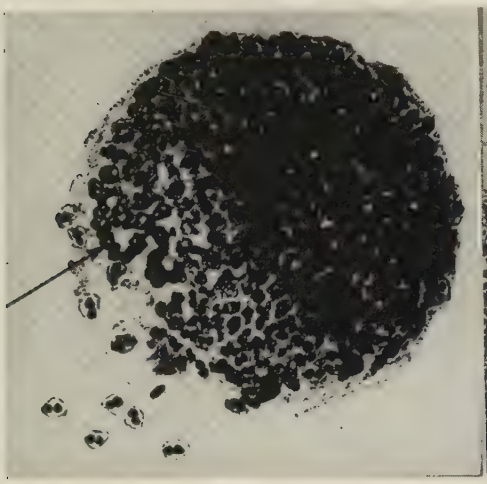


FIG. 1

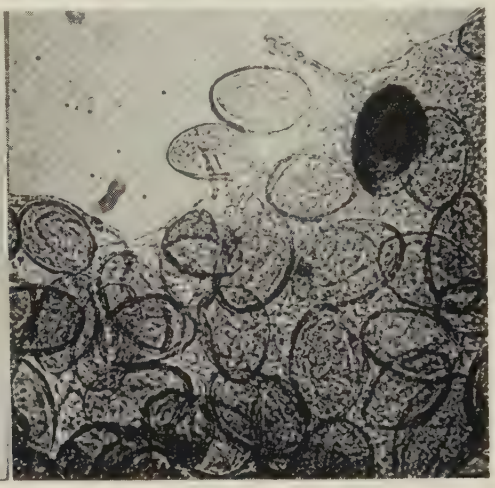


FIG. 2

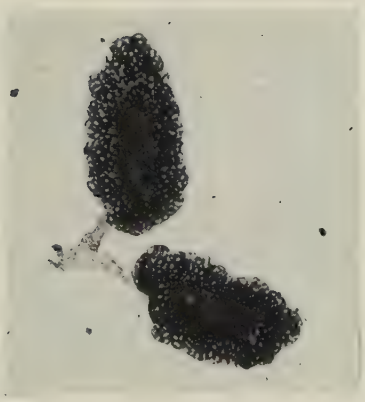


FIG. 3

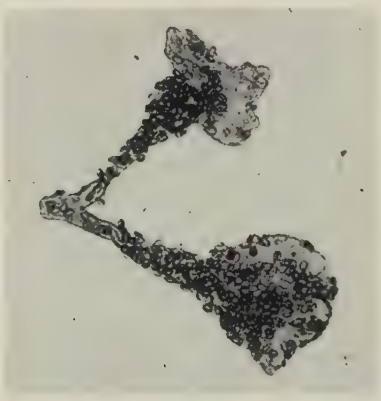


FIG. 4

FIG. 1. *Diplocystis metselaari* n. sp., coelomic gregarine parasite of *Anopheles farauti* in N. Guinea. Numerous binucleated zygotes in gametocyst. Arrow indicates residual protoplasm.

FIG. 2. *Coelomomyces walkeri* n. sp., fungus parasite of *Anopheles tesselatus* in Java. Sporangia in the ovary.

FIG. 3. *Coelomomyces walkeri* n. sp. in ovaries of *Anopheles tesselatus*. No eggs, crowded with sporangia.

FIG. 4. *Coelomomyces walkeri* n. sp. in ovaries of *Anopheles tesselatus*. No eggs, few sporangia.

and does not show septa. Neither nuclei nor anastomoses could be clearly observed. In the interior of one ovary a fragment of mycelium showed a terminal dilatation of 33 by 25 micra, a young sporangium. These terminal thickenings have been fully described and figured by Keilin (1921) and Iyengar (1935). In one female the ovaries were so crowded with sporangia (fig. 3) that no place remained for the

accurate observation of a few yet visible hyphae; in the ovaries of the other female, containing few sporangia (fig. 4), no hyphae were found.

SUMMARY

1. Papers on the occurrence of trematodes in *Anopheles* mosquitoes, on their larval development and on their development into the adult fluke *Pneumonoeces variegatus* Rud. in the lungs of the frog, have been reviewed, because they have been lost in the literature.

2. A gregarine *Diplocystis metselaari* n. sp. has been described, found by Metselaar in female *Anopheles farauti* in Dutch New Guinea. For the species, found by Johnson (1902) in *Anopheles maculipennis,* the name *Diplocystis johnsoni* n. sp. is proposed.

3. A fungus parasite, *Coelomomyces walkeri* n. sp., has been described from two female *Anopheles tesselatus,* caught in Indonesia. This species is identical with type 1 of this genus found by Walker in *Anopheles gambiae* in Africa.

4. Type 4 of *Coelomomyces* found by Walker in the same mosquito and the species observed by Manalang in common species of *Anopheles* in the Philippines are denominated *Coelomomyces ascariformis* n. sp.

REFERENCES[1]

BHATIA, B. L. 1930 Synopsis of the genera and classification of Haplocyte Gregarines. Parasitology **22:** 156–167.

BRESSLAU, E. L. AND BUSCHKIEL, M. 1919 Die Parasiten der Stechmückenlarven. Biol. Centralbl. **39:** 325–336.

CHRISTOPHERS, S. R. 1952 The recorded parasites of mosquitoes. Riv. di Parassit. **13:** 21–28.

CUÉNOT, L. C. M. J. 1901 Recherches sur l'évolution et la conjugaison des grégarines. Arch. de Biol. **17:** 581–652.

GANAPATI, P. N. AND TATE, P. 1949 On the gregarine *Lankesteria culicis* (Ross), 1898, from the mosquito *Aëdes* (*Finlaya*) *geniculatus* (Olivier). Parasitology **39:** 291–294.

GIBBINS, E. G. 1932 Natural malaria infection of house-frequenting *Anopheles* mosquitoes in Uganda. Ann. Trop. Med. and Parasit. **26:** 239–266.

IYENGAR, M. O. T. 1935 Two new fungi of the genus *Coelomomyces* parasitic in larvae of *Anopheles.* Parasitology **27:** 440–449.

JOHNSON, H. P. 1902 A new sporozoan parasite of *Anopheles.* J. Med. Res. **7:** 213–219.

JONES, T. W. 1950 Anopheline larvae as intermediate hosts for larval Trematodes. Parasitology **40:** 144–148.

KEILIN, D. 1921 On a new type of fungus: *Coelomomyces stegomyiae* n. g., n. sp., parasitic in the body-cavity of the larva of *Stegomyia scutellaris* Walker (*Diptera, Nematocera, Culicidae*). Parasitology **13:** 225–234.

KÜNSTLER, J. 1887 *Diplocystis schneideri* (nov. gen., nov. sp.). Tablettes Zoologiques **2:** 25–66 (quoted by Cuénot).

LÉGER, L. AND DUBOSCQ, O. 1903 Sur les larves d'*Anopheles* et leurs parasites en Corse. C. R. Assoc. franç. pour l'avance. Sci., Sess. **1902:** 703–704.

MANALANG, C. 1930 Coccidiosis in Anopheles mosquitoes. Philipp. J. Sci. **42:** 279–281.

RAY, N. H. 1933 On the gregarine, *Lankesteria culicis* (Ross), in the mosquito *Aëdes* (*Stegomyia*) *albopictus* Skuse. Parasitology **25:** 392–396.

VAN THIEL, P. H. 1921 Over het voorkomen van *Distomum* bij Anopheles. Tijdschr. vergelijk. Geneesk. **6:** 307–313. (also in Acta Leidensia **1:** 276–282.)

———1922 Aanteekeningen over *Agamodistomum anophelis.* Tijdschr. vergelijk. Geneesk. **7:** 305–321. (Also in Acta Leidensia **1:** 283–299.)

———1925 Beiträge zur Kenntnis der Trematoden aus *Anopheles maculipennis Meig.* Beiheft (1) Arch. f. Schiffs- u. Tropen-Hyg. **29:** 396–400. (also in Acta Leidensia **1:** 300–305.)

———1930 Die Entwicklung von *Agamodistomum anophelis* zum *Pneumonoeces variegatus* Rud. Zentralbl. f. Bakteriol. Orig. **117:** 103–112. (Also in Acta Leidensia **5:** 238–253.)

[1] For nonquoted references, see the papers by van Thiel 1922, 1930.

———1953 Method and significance of the determination of age of female anophelines for basic entomological research in DDT house spraying. Docum. de Medic. Geograph. et Trop. **5**: 289–303.

WALANDOUW, E. K. 1934 Nematoden als bestrijders van *Anopheles* larven. Geneesk. Tijdschr. Nederl.-Ind. **74**: 1219–1224.

WALKER, A. J. 1938 Fungal infections of mosquitoes, especially of *Anopheles costalis*. Ann. Trop. Med. Par. **32**: 231–244.

WENYON, C. M. 1911 Oriental sore in Bagdad, together with observations on a gregarine in *Stegomyia fasciata*, the haemogregarine of dogs and the flagellates of house flies. Parasitology **4**: 273–344.

WUNDSCH, H. H. 1911 *Pneumonoeces asper* Looss und sein Verhältnis zu den Gattungsverwandten. Thesis, Berlin, 76 pp.

MICROCOTYLE MACRACANTHA N. SP., A MONOGENETIC TREMATODE FROM THE GULF OF CALIFORNIA, WITH A REDESCRIPTION OF *AMPHIBDELLOIDES MACCALLUMI* (JOHNSTON AND TIEGS, 1922) PRICE, 1937

CLAUDE G. ALEXANDER

Department of Zoology, University of California, Los Angeles

In material collected during an extensive survey of helminth parasites from fishes of the Gulf of California, made in April, 1953, by Dr. Clark P. Read, a monogenetic fluke belonging to the genus *Microcotyle* van Beneden and Hesse, 1863, was present. The worms, which are from the gills of a mullet, *Mugil cephalus* L., are believed to differ sufficiently from the 76 species previously described to be considered representatives of a new species. The present paper deals with its description and with a redescription of *Amphibdelloides maccallumi* (Johnston and Tiegs, 1922) Price, 1937, from the gills of an electric ray, *Torpedo californica* Ayres, collected by Miss Anita Daugherty, California Dept. of Fish and Game, Marine Fisheries Branch, Terminal Island, California, in July, 1953, off the coast of southern California.

The author is indebted to Miss Daugherty and to Dr. Boyd W. Walker for his assistance in making the material from the California coast available for examination. Grateful acknowledgment is also made to Mr. Joseph Sefton for his generous loan of facilities during the Gulf survey, and to Dr. E. W. Price for loan of the type material of *Amphibdelloides maccallumi* from the U.S.N.M. Helminthological Collection, and for his examination, and opinion regarding, the status of the specimens of this species described herein. This study was carried out under the direction of Dr. Clark P. Read.

Eight specimens of the microcotylid were recovered from the host's gills, fixed in alcohol-formalin-acetic acid after relaxation in tap water in a refrigerator, stained with Grenacher's acidulated borax-carmine, and mounted in *toto*. Twenty-one specimens of the dactylogyrid were obtained from the gills of *T. californica* and fixed in a similar manner. Two specimens were sectioned frontally and stained with Harris' haematoxylin-eosin and Heidenhain's iron-haematoxylin, respectively; the remainder were stained with either Mayer's dilute haemalum or Delafield's dilute haematoxylin, or with coelestin blue B, according to the technique of Demke, 1952, and mounted *in toto*.

Received for publication, September 9, 1953.

Microcotyle macracantha n. sp.
Plate I, Figs. 1–4

Diagnosis: Slender worms, 0.900–1.160 mm. wide by 6.680–9.180 mm. long. Anterior end tapered to blunt point. Anterior suckers septate, each with double row of minute knob-like spines, approximately 52 in number, on anterior surface; suckers 0.036–0.049 mm. wide by 0.061–0.074 mm. long. Oral opening median, terminal; pharynx oval, 0.038–0.046 mm. wide by 0.053–0.076 mm. long. Esophagus relatively long, 0.357–0.608 mm. Ceca with numerous short, lateral diverticula; right cecum ending at anterior margin of haptor, left cecum extending to posterior end of haptor. Haptor 1.300–1.890 mm. long with 23–25 pairs of suckers in 2 rows. Largest suckers at anterior end of haptor, 0.093–0.144 mm. wide by 0.164–0.198 mm. long. Sucker sclerites consisting of 2 pairs of lateral, curved bars forming rim of sucker, and median, ventrally depressed piece with anterior end bifurcated into short wings; short accessory piece joining median piece to anterior rim-bar; anterior wings of posterior rim-bar curving ventrally. Genital aperture median, ventral, at point of intestinal bifurcation, 0.535–0.664 mm. from anterior end. Genital atrium enclosed by 15–20 pairs of large recurved spines; spines on each side consisting of group of 5–7 anterior hooks in single row, and 10–13 posterior hooks in double row; atrial spines 0.056–0.066 mm. in length. Testes small, numerous (152–213), transversely elongated, closely packed, chiefly intra-cecal, extending from ovary nearly to posterior end of vitellaria. Ovary median, about one-fourth the length of body from anterior end, in shape of flattened question mark; uterus highly coiled, extending in midline from ovary to genital aperture. Vitellaria consisting of numerous small follicles filling spaces between intestinal diverticula from about midway between intestinal bifurcation and ovary nearly to haptor. Vitelline ducts joining and entering vitelline reservoir in concavity formed by ovary. Eggs not present.

Host—*Mugil cephalus* L.

Location—Gills.

Locality—Coyote Bay (Concepcion Bay), Gulf of California, Baja California, Mexico.

Holotype—U.S.N.M. Helm. Coll. No. 49036.

Paratype—U.SN.M. Helm. Coll. No. 49037.

Additional specimens deposited in the helminthological collection, Dept. of Zoology, University of California, Los Angeles.

Reference to the descriptions of the species of *Microcotyle* by Sproston (1946) and of two new species since then (Pearse, 1949) shows that the present form differs from most other members of the genus in the relatively large size of the atrial spines. Two species have been described from *Mugil cephalus: M. mugilis* Vogt, 1878, from Roscoff and *M. cephalus* Abdel Azim, 1939, from Alexandria, Egypt. The present species resembles the latter form most closely, but differs in several respects. Although Abdel Azim (1939) did not indicate the size of the atrial spines in his description, he stated that only the anterior pair of spines possesses recurved points, while all those of *M. macracantha* have recurved points. In addition both intestinal ceca extend throughout the length of the haptor in *M. cephalus,* while in *M. macracantha* the right cecum terminates at the anterior end of the haptor. Other differences are the septate, armed anterior suckers in *M. macracantha* as opposed to non-septate, unarmed suckers in *M. cephalus,* and the somewhat smaller number of testes in the latter species.

Amphibdelloides maccallumi (Johnston and Tiegs, 1922) Price, 1937
Plate II, Figs. 1–5

Description: Slender worms, 0.330–0.570 mm. wide by 2.210–2.960 mm. long. Cephalic glands forming heavily staining masses on each side posterior to oral aperture, continuous across body anterior to oral aperture, opening to outside by 3 pairs of head organs at anterior tip of body. Oral aperture median, ventral, 0.224–0.280 mm. from anterior tip of body. Pharynx spherical, 0.100–0.127 mm. in diameter. Esophagus short, ceca bifurcating almost immediately behind pharynx, ending blindly at posterior tip of vitellaria. Haptor not lobed, but with 5 lateral and 1 posterior pairs of projections bearing 12 of 14 marginal hooklets; haptor 0.273–0.415 mm. wide; marginal hooklets about 9 μ long. Large haptoral hooks 0.127–0.182 mm. long, supported by single cuticular bar, 0.013–0.024 mm. wide by 0.062–0.082 mm. long; posterior edge of bar thickened, tubercular in appearance; lateral margins extending forward as short wings, forming

arms of broad 'V.' Genital aperture median, ventral, immediately behind intestinal bifurcation. Penis stylet slender, cuticularized, tubular, 0.140–0.182 mm. long; supported by 2 accessory pieces, 1 ending in curved tip, the other with 3 terminal points; accessory pieces 0.102–0.137 mm. long. Testis median, narrow, extending about three-fourths the distance to posterior end of vitellaria, connected by short vas deferens to large seminal vesicle. Seminal vesicle surrounded by mass of prostatic glands. Two prostatic vesicles present; ducts from seminal vesicle and prostatic vesicles converging and emptying into proximal end of penis stylet. Vitellaria consisting of wide band of loose follicles on each side ventrally, extending from anterior tip of ovary to posterior end of body; follicles continuous across body on ventral side at posterior end and continuous across body on dorsal side for entire body length. Ovary median, mostly pretesticular, slightly curved. Uterus extending from Mehlis' gland to genital aperture as thin, straight tube, slightly widened distally about half its length. Vagina consists of slender, cuticularized tube expanded at external exit on dorsum of body at level of anterior tip of testis; vaginal opening on right side of body, surrounded by sucker-like, muscular depression 0.122–0.137 mm. in diameter. Seminal receptacle large, median, extending from region of Mehlis' gland to level of anterior tip of testis.

Host—*Torpedo californica* Ayres.

Location—Gills.

Locality—Cortez Bank, off the coast of southern California. Two specimens deposited in the U.S.N.M. Helm. Coll. as Nos. 49038 and 49039.

Additional specimens deposited in the helminthological collection, Dept. of Zoology, University of California, Los Angeles.

These specimens are believed to constitute the first record of this species, the only one so far described in the genus, from the Pacific Ocean and to add a new host record. Since a thorough study of the reproductive system was made, and since a few points of difference from Price's (1937) description were noted in both the present material and the type specimens, a redescription of the species was considered desirable.

Literature Cited

Abdel Azim, M. 1939 Note sur *Microcotyle cephalus* n. sp., ectoparasite des arcs branchiaux de *Mugil cephalus*. Ann. Parasitol. **17**: 17–20.

Demke, D. D. 1952 Staining and mounting helminths. Stain Tech. **27**: 135–139.

Pearse, A. S. 1949 Observations on flatworms and nemerteans collected at Beaufort, N. C. Proc. U. S. Nat. Mus. **100**: 25–38.

Price, E. W. 1937 North American monogenetic trematodes. I. The superfamily Gyrodactyloidea. J. Wash. Acad. Sci. **27**: 146–164.

Sproston, N. G. 1946 A synopsis of the monogenetic trematodes. Trans. Zool. Soc. London **25**: 185–600.

Explanation of Plates

Abbreviations Used

a—accessory copulatory pieces
m—mehlis' gland
o—ovary
p—penis stylet
pg—prostatic gland
pv—prostatic vesicle
sr—seminal receptacle
sv—seminal vesicle
t—testis
u—uterus
v—vagina
y—vitellaria
yd—vitelline duct

All drawings except Fig. 1, Plate II, made with aid of camera lucida.

Plate I

Microcotyle macracantha n. sp.

Fig. 1. Entire worm, ventral view. 2. Anterior end, showing anterior suckers. 3. Spines of genital atrium. 4. Haptoral skeleton.

PLATE I

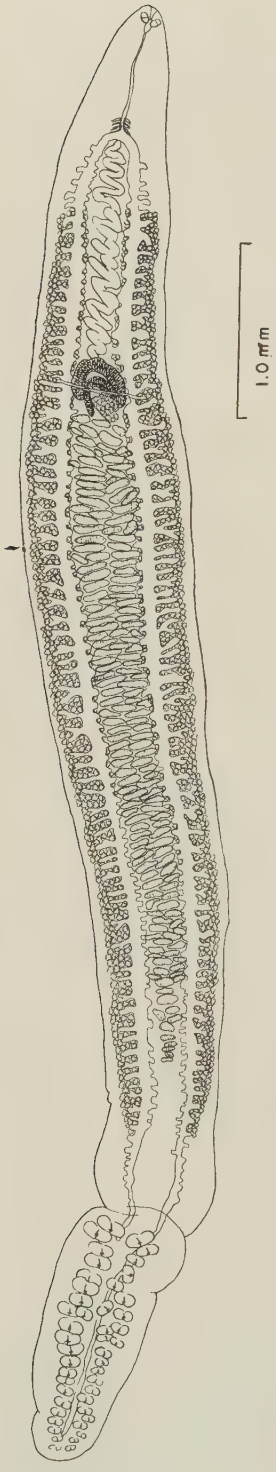


FIG. 1

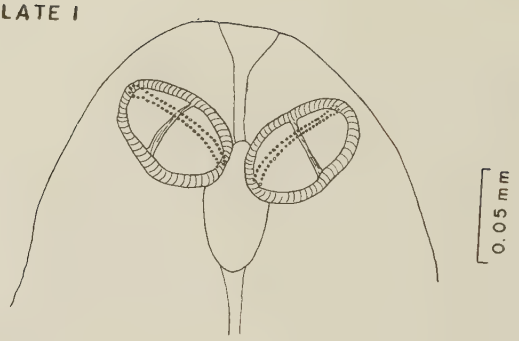


FIG. 2

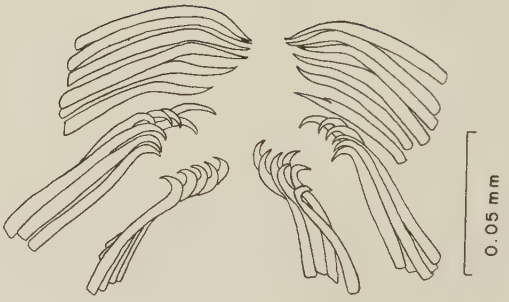


FIG. 3

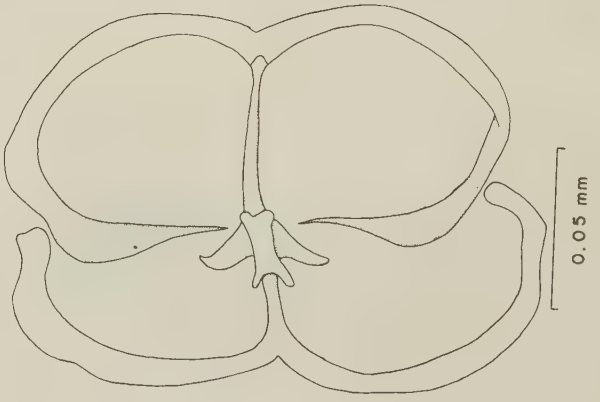


FIG. 4

PLATE II

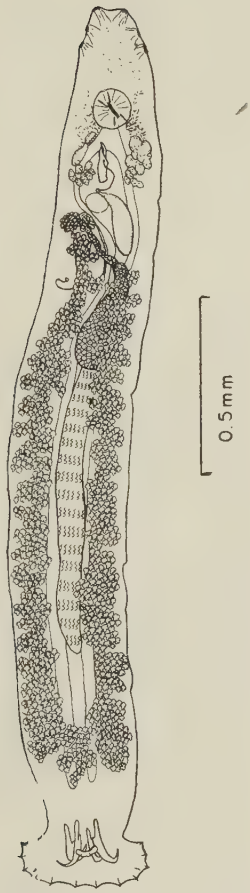


Fig. 2

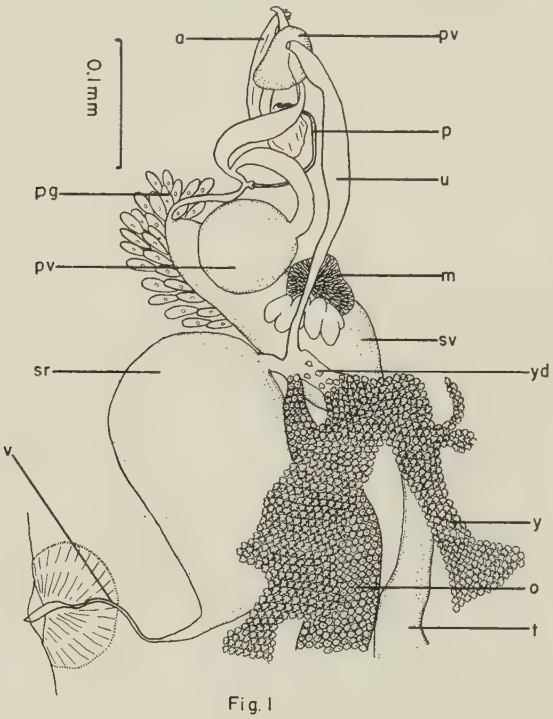


Fig. 1

Fig. 5

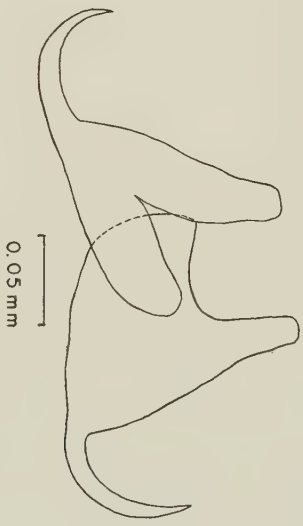


Fig. 4

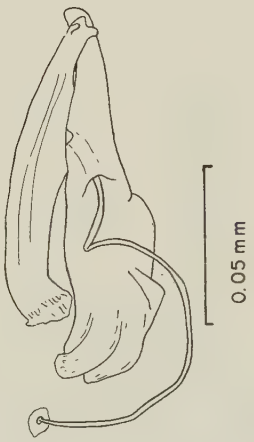


Fig. 3

PLATE II

Amphibdelloides maccallumi (Johnston and Tiegs, 1922) Price, 1937

FIG 1. Reproductive system. Reconstructed from serial frontal sections. 2. Entire worm, ventral view. 3. Penis stylet and accessory pieces. 4. One pair of large haptoral hooks. 5. Supporting bar.

STUDIES ON THE MORPHOLOGY AND LIFE CYCLE OF *NEOECHINORHYNCHUS EMYDIS* (LEIDY), AN ACANTHOCEPHALAN PARASITE OF THE MAP TURTLE, *GRAPTEMYS GEOGRAPHICA* (LE SUEUR)[1]

WILLIAM B. HOPP[2]

Department of Biological Sciences, Purdue University
Lafayette, Indiana

INTRODUCTION

In 1946, the writer reported a juvenile acanthocephalan obviously of the genus *Neoechinorhynchus* from cysts in the foot of the viviparid snail, *Campeloma rufum,* collected from the Tippecanoe River near Lafayette, Indiana. In a search for possible definitive hosts, turtles of various species were collected from that stream over a period of several years and examined for acanthocephalans. Negative results were obtained from all *Chrysemys picta marginata, Amyda ferox spinifera, Chelydra serpentina,* and *Sternotherus odoratus* examined. However, most specimens of *Pseudemys scripta elegans* and *Graptemys geographica* proved to harbor numerous acanthocephalans, all of which were at first considered to be *Neoechinorhynchus emydis* (Leidy, 1851) the only species of the genus described from turtles. Later, critical study revealed that the two hosts harbor different species of *Neoechinorhynchus.* These findings are contrary to the view of Van Cleave and Bullock (1950) who were of the very definite opinion that *N. emydis* is the only species of its genus parasitizing North American turtles. Their emphasis on this point may have been prompted by a personal communication in which they were informed of the writer's findings.

For reasons that will be given in the discussion, it is held that the form occurring in *Graptemys geographica* is *N. emydis* while the one that the writer has found in *Pseudemys scripta elegans* is another species of *Neoechinorhynchus* and will be considered in a separate paper. The present report deals with observations made on the life history of *N. emydis* after it was evident that the juvenile occurring in snails developed into the adult of that species of *Neoechinorhynchus.*

HISTORICAL REVIEW

Numerous observations bearing on the life histories of the Acanthocephala are reported in the literature, but relatively few cycles have been demonstrated experimentally. Since a comprehensive summary of the literature on that subject was given by Ward (1940), a review of life history studies since that time will suffice here. Yamaguti and Miyata (1942) gave a detailed account of the development of *Moniliformis dubius* in the intermediate host, *Periplaneta americana.* Moore (1946a) investigated the life history of *M. dubius* further under both natural and experimental conditions. He found that the acanthor penetrated the gut wall of *P. americana,* and after a period of 10 to 12 days of development while attached

Received for publication, November 4, 1953.

[1] Based on a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, Purdue University, August, 1953. This study was directed by Professor R. M. Cable.

[2] Present address, Department of Biology, Eastern Kentucky State College, Richmond, Kentucky.

to the wall, the acanthella dropped into the body cavity and continued its development there. Infective juveniles were present 7 to 8 weeks after infection. In another study Moore (1946b), gave a detailed account of the life cycle of *Macracanthorhynchus ingens,* a parasite of *Procyon lotor lotor.* He found that the basic pattern of development followed that of *M. hirudinaceus* closely but suggested that *M. ingens* may use *Rana pipiens* as a transport host. There is no information as to a transport host for *M. hirudinaceus.*

DeGiusti (1949) reported the life cycle of *Leptorhynchoides thecatus.* He found that the acanthor penetrated the intestinal epithelium of its experimental host, *Hyalella azteca,* and localized between the epithelium and the serosa. After 14 days of development attached to the gut, the acanthella separated from a residuum of the acanthor and completed its development to the infective juvenile free in the hemocoele of the cockroach. Thirty-two days elapsed from the time the amphipod ingested the egg until the resulting juvenile was capable of infecting the experimental definitive host, *Ambloplites rupestris.* Reish (1950) found juveniles of *Polymorphus kenti* in the sand crab, *Emerita analoga,* and reported preliminary studies on the life cycle of that parasite.

The only life cycle of a species of *Neoechinorhynchus* reported in any detail to date is that given by Ward (1940) for *N. cylindratus* from fishes. She found that the eggs of *N. cylindratus* escape with the feces of the definitive host, *Huro salmoides,* and are eaten by ostracods, *Cypria* (*Physacypria*) *globula,* in which the embryos develop to a stage closely resembling the adult worm except in size and maturity. The infected ostracods are eaten by bluegills, *Lepomis pallidus,* in the liver of which the juvenile worms localize and encyst. When the infected bluegills are eaten by bass, the parasites develop to maturity in the intestine. Ward was of the opinion that the bluegill serves in a more essential capacity than that of a mere transport host, but had no experimental evidence either for or against that view.

Some scattered observations have been reported concerning the life cycle of *N. emydis.* Whitlock (1939) reported what probably is the juvenile of that acanthocephalan from two species of snails, *Campeloma decisum* and *Pleurocera acuta,* in Michigan. Such juveniles were reported later from *Campeloma rufum* in Indiana by Hopp (1946) and from the same snail and *Ceriphasia semicarinata* in Kentucky by Lincicome and Whitt (1947). The form and arrangement of the proboscis hooks of the juvenile and presumably the adult of *N. emydis* was described by Lincicome (1948).

MATERIALS AND METHODS

Morphological details of both adult and juvenile worms were determined from specimens placed in water until the proboscis was everted and not withdrawn when touched. They were then fixed in Bouin's fluid, stained with hematein, and mounted in damar.

Eggs, or shelled embryos, were obtained from both the body cavity of adult females and the feces of turtles. At first, only eggs from the latter source were used in attempts to infect intermediate hosts, but it was later found that intermediate hosts were as susceptible to eggs removed from large female worms as to those voided in the host's feces. Since the eggs were too heavy to be levitated

by $ZnSO_4$ flotation, eggs in feces were concentrated by mixing with water and centrifuging. When treated in this manner, eggs were found to concentrate beneath most other sediment.

Snails were collected from mud banks of the Tippecanoe River, and observed for acanthocephalan cysts, which were easily seen with the naked eye as opaque spots in the expanded foot. Many snails were dissected in order to observe the distribution of the cysts, and the juvenile worms were removed from them for study, both alive and as fixed and stained whole mounts. Serial sections were prepared of the encysted juveniles *in situ* to observe their relationship to the host's tissues.

Ostracods were obtained from the Tippecanoe River and either examined for natural infections or used to start cultures, one of which was maintained over a year in the laboratory. Dissection of the ostracods proved to be a difficult and tedious process because of their small size, the shell averaging approximately 0.55 mm. in length. Furthermore, when the animal is disturbed, the valves of the shell are held tightly closed. The method finally used routinely after anaesthesia was abandoned was to place a single ostracod in a small drop of water on a microscope slide and carefully crack the shell with the edge of another slide. Then, with fine needles, the body of the animal was carefully separated from the shell and dissected.

Because of the minute size and fragility of the acanthor and acanthella stages, their handling and preparation for study involved difficulties. Because serum films, micropipette transfers, and other techniques yielded indifferent results, most of the observations were made on fresh material, either unstained or stained with red or methyl green-pyronin. Serial sections of exposed ostracods were made to study the development of the parasites *in situ* and to determine their position in the host.

Attempts to induce "artificial hatching" of eggs by the methods given by Manter (1927, 1928) and Moore (1942) to obtain acanthors for study were all unsuccessful. These investigators were able to induce "hatching" in eggs of certain other species, although Moore tried his method on eggs of *Neoechinorhynchus emydis* and *Centrorhynchus* sp. without success.

OBSERVATIONS AND DISCUSSION

Experimental Determination of the Life History

After encysted juvenile acanthocephalans were found in the foot of *Campeloma rufum,* infected snail tissue was fed to each of 6 specimens of *Chrysemys picta marginata* over a period of a month. The remainder of the lot, all collected from the same pond, had been killed and examined without finding acanthocephalan parasites of any sort and since the 6 turtles used for feeding experiments had no eggs in the feces they were presumed to be free of infection. After feeding the turtles were sacrificed at intervals, and young adults of the acanthocephalan encysted in the tissues of the snail were recovered from the intestines. Similar feeding experiments were performed with *Amyda ferox spinifera, Sternotherus odoratus, Terrapene carolina, Rana catesbiana,* and several species of catfish. A few young acanthocephalans were recovered from a specimen of *A. ferox spinifera,* but all other turtles and fishes were negative.

Since a turtle was indicated as the definitive host, it was a simple matter to find such a host naturally infected with adult worms that were comparable to those experimentally produced in *Chrysemys picta marginata.* The map turtle, *Graptemys geographica,* is abundant in the Tippecanoe River, and practically every specimen larger than a juvenile was found to harbor some of the worms. They were often present in such numbers that when the intestine was opened, it was coated with the parasites. Since mollusks constitute a major portion of the diet of *G. geographica,* there was little question that this turtle was the natural definitive host of the acanthocephalan whose juvenile stage had been found in the foot of *Campeloma rufum.*

Although all known life cycles of acanthocephalans include an arthropod as the first intermediate host, it at first seemed possible that in this instance the snail might obtain the parasite directly by ingesting the acanthocephalan eggs. This possibility was accordingly tested thoroughly and at length, but only negative results were obtained.

As it then seemed evident that the snails became infected in some manner other than by eating the eggs of the worm, other possibilities were sought. Because Ward (1940) had shown that an ostracod served as the first intermediate host of another species of *Neoechinorhynchus,* a search for ostracods was made in the habitat of infected snails. A minute species identified as *Cypria maculata* Hoff, 1942, was found living in the flocculant layer on the mud at the bottom of the river and several hundred specimens were collected and examined for natural infections. A juvenile worm unmistakably of the genus *Neoechinorhynchus* was recovered from the 168th ostracod and a low incidence of late acanthellas and juveniles was found in subsequent examinations. The next step obviously was to expose ostracods to eggs and this was done in small glass containers of water in which ostracods were allowed to feed on eggs for varying periods of time. It was found that the survival rate of ostracods was greater if they either were not exposed to massive numbers of eggs or were not allowed to feed for longer than 2 hours. At the end of that time, the ostracods were removed to other containers and dissected at intervals to obtain the various developmental stages. On subsequent examination, the incidence of infection in experimental ostracods was found to be so high (almost 100%) as to preclude any possibility that all of the parasites recovered were present as natural infections in the crustaceans at the time of collection. Furthermore, when examined a few hours after exposure to eggs, the intestine of the ostracod was found to contain newly-hatched acanthors moving slowly in the lumen. As such motility is lost within a few hours after emergence from the egg, the acanthors observed must have been from recently ingested eggs.

To date, experiments designed to infect snails with stages from the ostracod have not been successful, but the entire cycle seems evident, since it was carried from the snail through to the adult in the turtle, and from that host back to the ostracod. *Campeloma rufum* and the ostracod have the same habitat, and in plowing through the mud and feeding on debris, it seems evident that the snail would ingest numerous ostracods. This is suggested not only by the feeding habits of the snail but also by the activities of the ostracods. When brought into

the laboratory, they tend to stay in or near the flocculant top layer of mud and, upon the slightest disturbance, plunge into the protection of the mud.

The question remains as to whether the snail is an essential host in the life cycle. The necessity of a second intermediate host has been questioned in the life cycles of acanthocephalans in general. It has been argued that, should an arthropod intermediate host be ingested either by an animal unsuitable for further development of the parasite or by the natural definitive host before stages in the arthropod are infective, the parasites may escape from the digestive tract and continue development in the tissues of the second animal. In the case of *Neoechinorhynchus emydis,* this animal would be the snail and, according to the views held by some investigators, would not be an essential host in the life cycle. Instead, the snail would serve as an auxiliary or transport host, which would be of some aid to the parasite in that young stages which might otherwise be lost would survive as a potential source of infection of the definitive host. This argument is based primarily on the fact that a second intermediate or even a transport host is not found in the life cycles of some better known thorny-headed worms. The possibility that this may be the case in the life cycle of *N. emydis* has not been excluded and is being investigated further by feeding ostracods containing infective juveniles to young geographic turtles obtained shortly after hatching and reared in the laboratory. To date, 2 such turtles have been used for this purpose. They were fed a number of infected ostracods over a period of several weeks and then examined but no acanthocephalans were present in the intestine. Furthermore, minute scrutiny of the tissues bit by bit revealed no juvenile parasites. Turtles of the same age, when fed juveniles in snail tissues, became heavily infected with the adults of *N. emydis.* Although these results indicate that the snail serves in a more essential capacity than that of a transport host, further experiments are necessary to decide this matter.

The most important point would seem to be the method whereby the host obtains the majority of its parasites. Ward (1940) showed that the ostracod intermediate host of *Neoechinorhynchus cylindratus* was ingested by bluegills and the worms encysted in the liver in large numbers. She pointed out that it was much more likely that the bass, which serves as the definitive host, obtains massive infections by ingesting bluegills than by eating ostracods. The same situation would seem to be true in the case of *N. emydis,* most of which the turtle undoubtedly obtains by the ingestion of snails as the major item of its diet.

Incidence of Natural Infections

Definitive Host.

Natural infections with *Neoechinorhynchus emydis* were found in 29 of 32 map turtles, *Graptemys geographica.* The number of worms recovered from these turtles ranged from 2 to 243 per host with an average of 73. The 3 uninfected turtles and 2 others that yielded only a few worms were all immature. In general, the larger the turtle, the heavier the worm burden proved to be.

First Intermediate Host.

From 1,330 ostracods collected from the Tippecanoe River during the summer months and examined for natural infections, a total of 9 infective juveniles and 2 late acanthellas were recovered. As each infected ostracod harbored but a single

parasite, the incidence was 0.83%. The 2 late acanthellas were well-developed and capable of considerable movement although unable to evert the proboscis. The body wall showed a marked wrinkling, a condition noted also in the juveniles which otherwise resemble the adult *N. emydis* closely, except for size and maturity.

"Second Intermediate" Host.

In a series of 400 *Campeloma rufum* collected from the Tippecanoe River during several summers, 349, or 87%, were found to contain 1 or more encysted juveniles of *N. emydis*. Although these cysts were found at the base of the tentacles, near the mouth, and in the mantle, by far the greatest number occurred in the tissues of the foot. A weak cyst wall that ruptured easily was present and as a rule, only 1 worm was found in each cyst, although 2 were present in a few cases. All worms were infective juveniles, to judge from the extent of development and their size. Occasionally juveniles were found free in dishes containing snails; presumably the worms had been released by the spontaneous rupture of cysts, most of which were located superficially in the tissues. As many as 8 cysts occurred in the foot of a single snail. In an examination of other species of snails from the same region, a few specimens of *Pleurocera acuta* were found to be infected, but the number was so small as to indicate that *P. acuta* plays a minor role in the life history. To judge from the high percentage of natural infections, *Campeloma rufum* is the principal source of infection for *Graptemys geographica*.

Description of Stages in the Life Cycle

Van Cleave and Bullock (1950) gave a detailed description of the praesoma in the adult. Aside from that region, however, their paper deals in only a general way with the morphology of the worm which has never been adequately described. For that reason, a brief characterization and figures of the adult stage are included in this report. Unless otherwise stated, the body lengths given below exclude the praesoma and measurements of body width are from the lateral aspect at the widest region, usually in the vicinity of the first dorsal subcuticular nucleus. The proboscis lengths given represent the distance from the tip of the everted proboscis to a line connecting the basal hooks in the longest rows. All measurements are in millimeters.

Adults (Figs. 1–2, 7–10).

Neoechinorhynchus emydis possesses the diagnostic characteristics of its genus, viz., a globular proboscis with 3 circlets of 6 hooks each; body spines absent; subcuticula with a few giant nuclei; a single muscular layer in the proboscis receptacle; and the cement gland of the male consisting of a single syncytial mass with typically 8 large nuclei. The body of the living worm is white, becoming yellowish in fully gravid females, and is typically curved in extended specimens (Figs. 1 and 2). The proboscis is slightly broader than long, measuring 0.120 to 0.210 (av. 0.175) in length, and 0.180 to 0.230 (av. 0.216) in width. The proboscis hooks are in quincunxial arrangement; those of the anterior circlet are 0.065 to 0.107 in length with the lateral pair distinctly the largest and set back of a line connecting other hooks of the circlet. The hooks of the middle circlet are 0.042 to 0.059 in length and those of the basal circlet are 0.035 to 0.052 long. The longer of the two lemnisci contains two nuclei and the shorter a single nucleus. There are 6 subcuticular nuclei of

which 5 are in the mid-dorsal, and 1 in the mid-ventral line near the anterior end, as is characteristic of the family Neoechinorhynchidae.

Fifteen mature males (Fig. 8) ranged from 6.28 to 14.5 in length and 0.48 to 1.04 in width. The reproductive organs occupy approximately the posterior half of the body. The 2 testes are contiguous, in tandem, and immediately followed by the large, syncytial cement gland. Posterior to the cement gland is a globular cement receptacle, with a thin-walled cement tubule passing posteriorly to the bursa.

Fifteen mature females (Fig. 7) varied from 10.2 to 22.2 in length and 0.75 to 1.25 width. The genital pore is subterminal, opening on the ventral surface of the body. The ovarian balls are spherical or very slightly ellipsoidal, ranging from 0.040 to 0.055 in diameter.

Representative specimens; topotypes: (1) In author's collection, and (2) male and female in U.S. Nat. Mus. Helm. Coll., Nos. 37406 and 37407.

Egg or Shelled Embryo (Figs. 3–5, 11).

The fully developed egg of *N. emydis* varies little from 0.025 in length when measured in the fresh state, but may be from 0.018 to 0.022 long in whole mounts of mature females. The living egg is oval in cross section, measuring about 0.022 in width from what might be termed the frontal aspect (Figs. 3 and 11, upper left) and about 0.018 wide in "side" view (Figs. 5 and 11, lower right). There are three egg membranes: the outer shell, the inner fertilization membrane, and between them a middle shell membrane which is the most conspicuous and distinctive feature of the egg. Instead of enclosing the embryo as in many other acanthocephalan eggs, the middle membrane occupies only part of the space between the other 2 and resembles a vacuole more than anything else. In living eggs, this structure is about 0.013 wide and extends approximately half-way around the transverse circumference of the egg. Within the body of stained females, the characteristic appearance of the eggs is retained to some degree but is much less conspicuous than in fresh material. The structure of the middle shell membrane suggests that were it enlarged to surround the embryo, its edges would meet to form a raphe extending the length of the egg as described for certain other acanthocephala. However, such a raphe is usually said to extend around the long circumference of the egg and form a line along which the shell splits into 2 halves on hatching of the embryo.

The embryo or acanthor within the egg has an average length of 0.022 and a width of 0.011 in the fresh state. In its center is the embryonal nuclear mass or entoblast, the primordium of all future structures in the adult except the subcuticula and the lemnisci. The entoblast is situated in a clear space and is composed of a mass of small nuclei enclosing 1 larger nucleus. The peripheral region of the embryo is granular and contains 4 to 8 prominent bodies which may be the antecedents of the giant nuclei observed in later development.

In some respects, the above characterization of the adults and shelled embryos of *N. emydis* in in agreement with existing descriptions but in others it is not. Some points of disagreement may be attributed to differences in the preparation of material and its observation, but others are of such magnitude as to preclude explanation on that basis. For example, the maximum length of mature females given by Van Cleave and Bullock (1950) is almost twice that ever observed in the hundreds of specimens which the writer has taken from *Graptemys geographica,* but does

agree with the length of those which have been found repeatedly in *Pseudemys scripta elegans* from the same locality. Also, the acanthocephalan which Bravo-Hollis (1946) described as *N. emydis* from *Chrysemys ornata* in Mexico is significantly longer than *N. emydis* from *G. geographica* and even then Bravo-Hollis's description seems to have been based on contracted specimens. Furthermore, she gave measurements of embryos indicating a length–width ratio of 2.5 : 1 which cannot conceivably be harmonized with such observation on comparable material from *G. geographica.*

It therefore is evident that North American turtles harbor more than 1 species of *Neoechinorhynchus.* In fact, the 2 species occurring in *G. geographica* and *P. scripta elegans* taken from the same stream can be distinguished at a glance on the basis of the shelled embryos alone, even in whole mounts in which there may be considerable shrinkage and distortion of the eggs. This being the situation, it seems very likely that other species of turtles may harbor additional species of *Neoechinorhynchus.*

The identity of the species whose life cycle is here reported remains to be considered. Although Leidy's original description of *N. emydis* is vague and incomplete, some statements in his papers assume unexpected significance in the light of the writer's observations. In first describing the species, Leidy (1851) gave as the host *only* the geographic turtle which harbors the species here under consideration. Leidy also gave the length of the worm as up to "8 lines" (ca. 17 mm.) which is well within the limits observed in the writer's material from that host. Later, Leidy (1856) listed the species and gave some additional data which now appears to be of considerable importance: he named 3 additional species of turtles as hosts and recorded the length of the worm as being up to "14 lines" (almost 30 mm.) which is significantly greater than the maximum observed in the writer's specimens from *G. geographica.* Van Cleave and Bullock (1950) stated, "About 30 mm. is average size for fully mature females in the northern part of the geographical range but in southern Illinois and southward . . . females at times attain the greater length of 40 mm. . . ." The host of such large individuals was not named. The species which the writer has found in *P. scripta elegans* in northwestern Indiana attains such a length but it definitely is not the species that occurs in *G. geographica* from the same habitat. As Van Cleave and Bullock were convinced from their study of a large collection that turtles harbor but 1 species of *Neoechinorhynchus,* it is the writer's opinion that from 1856 when Leidy listed additional hosts and a greater length of the worm, investigators have treated as a single species forms representing at least 2 distinct species. In view of Leidy's accounts, the species of *Neoechinorhynchus* from *G. geographica* is here considered to be the species that Leidy originally described from that host and named *N. emydis.* Another paper will deal with the species which has been found to occur in *P. scripta elegans.*

Acanthor (Figs. 6, 12).

The emergence of the embryo from the egg was not observed, but actively motile acanthors were found upon the dissection of ostracods 8 to 12 hours after exposure to eggs. Because the acanthors were still within the lumen of the intestine and empty egg cases were present, it was evident that the embryos had been free but a short time. Their activity consisted of an apparently aimless, lunging motion,

with the anterior and posterior regions extending and contracting alternately. Such movements within the intestine of the ostracod would evidently result in inching forward, but no such progress was observed in a drop of water on a slide. An immediate increase in the size of the embryo upon hatching seems to occur in this species as has been reported for other acanthocephalans (Manter, 1928; Ward, 1940). The free acanthor, when extended, averaged 0.033 in length and 0.014 in width, measurements which are significantly larger than those of the embryos before release. The body of the acanthor is spindle-shaped and somewhat blunt at the anterior end (Fig. 6). Little change is observed in the entoblast or the space around it, but 1 of the large nuclei of the peripheral region migrates to the more pointed posterior end and becomes distinctly vesicular with a conspicuous nuclear membrane, as do some of the other nuclei of the peripheral region. The anterior end of the acanthor bears a thin-walled, sac-like invagination, and rows of extremely minute spines.

The acanthor penetrates the wall of the intestine within 12 to 24 hours after the ostracod has been exposed and appears to enter the body cavity immediately, without a period of attachment to the gut wall of the intermediate host. Second stage acanthors and acanthellas in all stages of development were found free in the body cavity, with no indication of attachment to any tissue or organ. Thus the entire acanthor contributes to the formation of the acanthella, no part being lost as a consequence of attachment.

About 2 days after exposure of the ostracod, "second stage" acanthors (Fig. 12) in the body cavity lack all signs of motility. There is a slight increase in size, the larvae averaging 0.038 in length and 0.015 in width. Little change in shape is to be seen, and the internal structures are essentially unchanged except for the disappearance of the anterior sac-like invagination of the active acanthor.

Acanthellas (Figs. 13–17).

After 3 days of development, the acanthella assumes an oval shape (Fig. 13), having increased in width to 0.024 without any appreciable lengthening of the larva. The entoblast and the clear area around it have increased somewhat in size, and 4 giant nuclei with distinct membranes are visible.

Four days after exposure, a further increase in size is noted, the acanthella measuring 0.047 in length and 0.031 in width. Six giant nuclei are visible.

Acanthellas removed from ostracods 5 days after exposure measure approximately 0.057 in length and 0.048 in width. The entoblast shows an accompanying increase in size, and 6 giant nuclei are visible.

By the sixth day of development in the ostracod, the acanthella (Fig. 14) is 0.066 in length and 0.052 in width. Although there is no increase in the number of giant nuclei, 2 of the 6 are much larger than the others.

After 7 days of development, the acanthella (Fig. 15) has increased considerably in size, being 0.128 in length and 0.105 in width. Nine giant nuclei are visible, 2 of them decidedly larger than the rest. The entoblast has assumed a somewhat flask-shaped appearance, with a projection extending toward the anterior end of the larva. The individual nuclei making up the entoblastic mass are distinct.

By the 8th day, the acanthella has elongated considerably and assumed a blunt, sausage-shaped appearance (Fig. 16). The larva has grown to a length of 0.288

and a width of 0.083. Six of the giant nuclei, including the 2 larger ones, take up positions along the outer curve of the acanthella, 2 along the inner curve, and 1 at the anterior end. The entoblast has become elongate and differentiated.

By the tenth day, a further elongation of the acanthella has occurred, and the basic division of the worm into praesoma and body is established. The total length is approximately 0.38, the body 0.047 in maximum width, and the praesomatic region 0.080 in width. The body has a characteristic irregular, wrinkled appearance which persists in subsequent development. Along its length are bulges where the giant nuclei are located. Six of these are in the body region, and 2 are visible in the praesoma, with the ninth observed earlier not evident but presumed to be present. The acanthellas at this stage of development and for several days thereafter were so delicate that they were extremely difficult to handle without damage to them.

Acanthellas from ostracods dissected 12 days after exposure measure approximately 0.55 in length, 0.069 in maximum body width, and 0.104 in praesomatic width. When freed from the ostracod, the young worm tends to assume the curved form typical of the adult. The giant nuclei occupy the same position as in the adult worm: e.g., 5 in the mid-dorsal line and 1 in the mid-ventral line where prominent bulges are seen on the body surface.

Fourteen days of development result in an acanthella that shows its greatest advancement in the further differentiation of the praesomatic region. A slight decrease in total length is noted, the average being 0.42; this phenomenon probably is associated with the development of the muscular system which enables the worm to contract. The maximum width of the body is 0.070. The praesomatic region has an anterior protuberance containing 3 nuclei, those of the apical ring. Within the praesomatic region itself can be seen the future proboscis outlined by a clear space surrounding it.

By the 16th day, the acanthella again shows a slight decrease in total length to approximately 0.38 with an increase in maximum width to 0.10. Praesomatic development has advanced to a point that the proboscis is well-defined, the hooks are developing although they have not reached full size, and the proboscis is partially retractile. A feature often noted in this and subsequent stages is an extreme enlargement of 1 of the mid-dorsal giant nuclei, resulting in a prominent distortion of the body configuration. The significance of this feature is not known, although it occurred so constantly as to indicate that it is not an abnormality.

Individuals recovered on the 17th day (Fig. 17) are so well developed that they might seem to be infective juveniles. Their interpretation as late acanthella seems justified, however, for several reasons. They average 0.45 in length and approximately 0.10 in their greatest body width. An interesting feature is the extreme variation in the size of the hooks in the terminal circlet. The 2 lateral hooks measure 0.080 in length, while the other 4 measure 0.064. Lincicome (1948) reported a similar variation in size of the terminal circlet of hooks in both juveniles and adults. Although partial retraction of the proboscis is possible, the 2 lateral hooks are so large that their tips protrude. The giant nuclei remain very prominent, and the future sexual apparatus has made its appearance in the posterior region of the body, although it has not developed sufficiently to indicate the sex of the individual.

After 19 days of development in the ostracod, the acanthella has further increased in size to an average length of 0.71. The proboscis has been fully inverted into the body, and the completely formed hooks are visible within the proboscis receptacle. The proboscis evidently is incapable of protrusion at this time, since eversion could not be elicited by mechanical stimulation, pressure on the coverslip, or the imbibition of water, all of which cause the protrusion of the proboscis in the juvenile and the adult. The anterior region of the body is considerably enlarged due to the presence of the inverted proboscis, and the reproductive apparatus is developed to the extent that the sex can be readily determined.

Juveniles in the Ostracod (Figs. 18 and 19).

Completely developed infective juveniles (Fig. 18) are present in the ostracod 21 days after feeding on the eggs of *N. emydis*. The maximum length observed was 1.10, of which the praesomatic length accounted for 0.14. Although some wrinkling of the body wall is evident, it is not as pronounced as in earlier stages. The giant subcuticular nuclei are not as prominent, and they occupy the same position as in the adult worm. The proboscis is capable of full eversion and inversion. The 2 lateral hooks in the terminal circlet measure approximately 0.088 in length as compared to a length of 0.079 for the other 4 hooks in the same ring; the hooks of the middle circlet average 0.040 in length; and those of the basal circlet 0.037 in length. The immature reproductive structures are well developed. The minute size of the ostracod probably limits further growth of the parasite, which is folded and tightly packed within the body cavity.

Juveniles in the Snail (Figs. 19 and 20).

Only fully-developed juveniles (Fig. 19) were recovered from cysts in the foot of *Campeloma rufum*. The thin-walled cysts (Fig. 20), which are filled with what seems to be cellular debris, average approximately 1.0 in diameter. The worms are more or less coiled within the cyst. The total length of a series of 29 such juveniles ranged from 1.20 to 1.87 (avg. 1.44) of which the praesoma represented an average of 0.166. The maximum body width averaged 0.198. The 2 lateral hooks of the terminal circlet averaged 0.088 in length, and the other 4 0.083; the hooks of the middle circlet were 0.054 in length; and the hooks of the basal circlet were 0.047 in length. Except for the increase in size and the decrease in the wrinkling of the cuticula, little difference could be observed between juveniles from the ostracod and those from the snail (Fig. 19). It may be noted that an increase in the size of the hooks, especially in the middle and basal circlets, occurs after release from the ostracod.

SUMMARY

Neoechinorhynchus emydis is redescribed and an account of the life cycle is given. The egg is ingested by the ostracod, *Cypria maculata*, and hatches in the intestine, releasing a typical acanthor which enters the body cavity of the ostracod and develops to an unencysted juvenile in 21 days without a period of attachment to the host's intestine. Development within the ostracod was observed at frequent intervals and is described. Ostracods containing juveniles are ingested by snails, particularly *Campeloma rufum*, in which the worms enter and become encysted in the tissues, especially of the foot in which cysts are seen as opaque spots. Some

growth of the juvenile occurs within the snail which serves as the principal source of infection of the map turtle whose food is largely mollusks.

The discovery of another species of *Neoechinorhynchus* which occurs in *Pseudemys scripta elegans,* is much larger than *N. emydis,* and has entirely different eggs, suggests that existing descriptions of *N. emydis* are based on more than one species.

Literature Cited

Bravo-Hollis, M. 1946 *Neoechinorhynchus emydis* (Leidy, 1852); Van Cleave, 1913, parasito del intestino de *Chrysemys ornata.* An. Inst. Biol. Univ. Nac. México **17:** 187–192.

DeGiusti, D. L. 1949 The life cycle of *Leptorhynchoides thecatus* (Linton), an acanthocephalan of fish. J. Parasit. **35:** 437–460.

Hoff, C. C. 1942 The ostracods of Illinois, their biology and taxonomy. Ill. Biol. Mon. 19 (1, 2), 196 pp.

Hopp, W. B. 1946 Notes on the life history of *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of turtles. Proc. Indiana Acad. Sci. **55:** 183.

Leidy, J. 1851 Contributions to helminthology. Proc. Acad. Nat. Sci. Phila. (1850–1851) **5:** 205–209.

———1856 A synopsis of Entozoa and some of their ectocongeners observed by the author. Proc. Acad. Nat. Sci. Phila. **8:** 42–58.

Lincicome, D. R. 1948 Observations on *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of turtles. J. Parasit. **34:** 51–54.

——— and Whitt, A. 1947 Occurrence of *Neoechinorhynchus emydis* (Acanthocephala) in snails. Tr. Ky. Acad. Sci. **12:** 19.

Manter, H. W. 1927 Artificial hatching of the eggs of the thorny-headed worm of hogs. J. Parasit. **13:** 223.

———1928 Notes on the eggs and larvae of the thorny-headed worms of hogs. Tr. Am. Micr. Soc. **47:** 342–347.

Moore, D. V. 1942 An improved technique for the study of the acanthor stage in certain acanthocephalan life histories. J. Parasit. **28:** 495.

———1946a Studies on the life history and development of *Moniliformis dubius* Meyer, 1933. J. Parasit. **32:** 257–271.

———1946b Studies on the life history and development of *Macracanthorhynchus ingens* Meyer, 1933, with a redescription of the adult worm. J. Parasit. **32:** 387–399.

Reish, D. J. 1950 Preliminary note on the life cycle of the acanthocephalan, *Polymorphus kenti* Van Cleave, 1947. J. Parasit. **36:** 496.

Van Cleave, H. J. and Bullock, W. L. 1950 Morphology of *Neoechinorhynchus emydis,* a typical representative of the Eoacanthocephala. I. The praesoma. Tr. Am. Micr. Soc. **69:** 288–308.

Ward, H. L. 1940 Studies on the life history of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) (Acanthocephala). Tr. Am. Micr. Soc. **59:** 327–347.

Whitlock, S. C. 1939 Snails as intermediate hosts of Acanthocephala. J. Parasit. **25:** 443.

Yamaguti, S. and Miyata, I. 1942 Über die Entwicklungsgeschichte von *Moniliformis dubius* Meyer, 1933 (Acanthocephala), mit besonderer Berücksichtigung seiner Entwicklung im Zwischenwirt. Parasit. Lab. Kaiserl. Univ. Kyoto, 32 pp.

Explanation of Plates

All figures are of *Neoechinorhynchus emydis.* Figures 1–2 and 7–10 were drawn by microprojection, 3–6 are free-hand, and 11–20 are unretouched photomicrographs.

Abbreviations: AS, acanthor spines; AT, anterior testis; B, introverted bursa; CD, cement duct; CG, cement gland; E, egg within body cavity; FV, vacuole-like middle shell membrane; LA, uninucleate lemniscus; LB, binucleate lemniscus; OB, ovarian ball within body cavity; PT, posterior testis; U, uterus; UB, uterine bell; V, vagina.

Plate I

Fig. 1. Outline of gravid female.
Fig. 2. Outline of adult male.
Fig. 3. Egg in "frontal" view.
Fig. 4. Egg in polar view.
Fig. 5. Egg in "side" view.
Fig. 6. Acanthor from intestine of ostracod.
Fig. 7. Posterior end of adult female.
Fig. 8. Posterior end of adult male.
Fig. 9. Praesoma of adult male.
Fig 10. Anterior end of adult male.

PLATE I

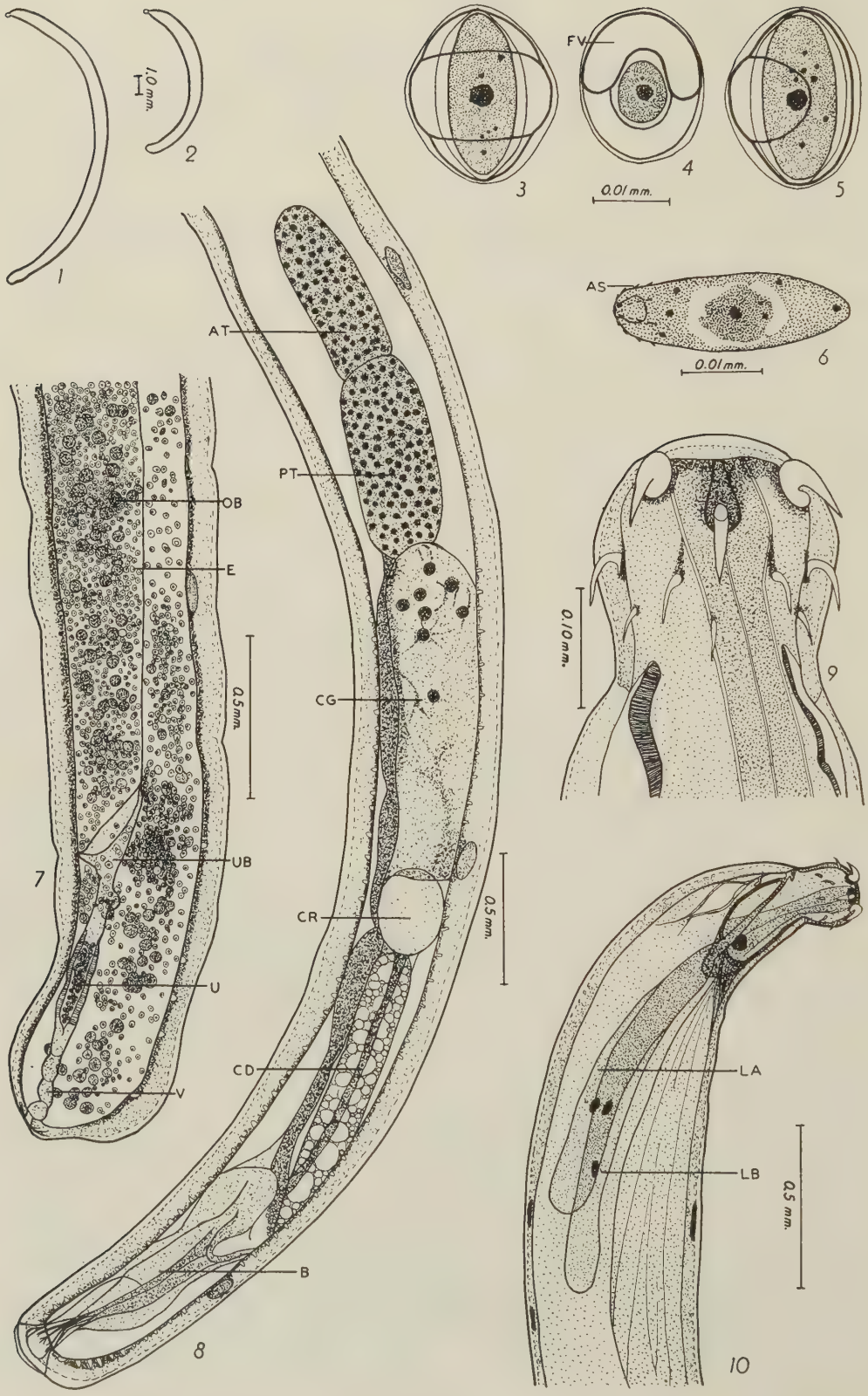

1.0 mm.
1
2
3
FV
4
0.01 mm.
5
AS
0.01 mm.
6
AT
PT
OB
E
0.5 mm.
CG
UB
7
CR
U
V
CD
0.5 mm.
B
8
0.10 mm.
9
LA
LB
0.5 mm.
10

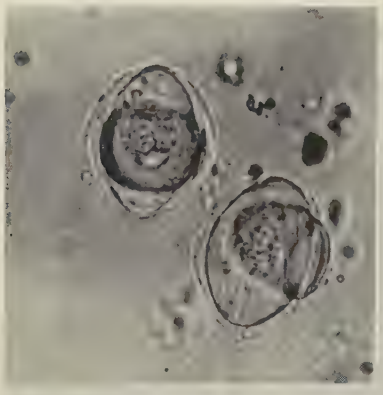
PLATE II

FIG. 11. Eggs in "frontal" (upper left) and "side" views (slightly less than 900 diameters).
FIG. 12. Inactive acanthor from body cavity of ostracod (250 diam.).
FIG. 13. Acanthella, three days (250 diam.).
FIG. 14. Acanthella, six days (250 diam.).
FIG. 15. Acanthella, seven days (250 diam.).
FIG. 16. Acanthella, eight days (250 diam.).

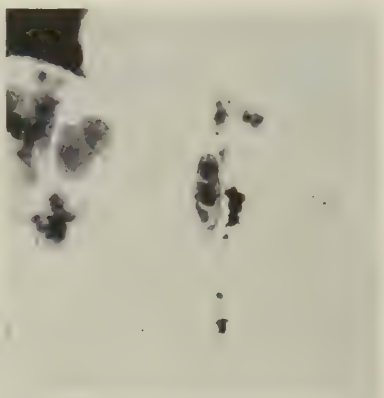
PLATE III

FIG. 17. Acanthella, 17 days (300 diam.).
FIG. 18. Juvenile (male) from ostracod (135 diam.)
FIG. 19. Fully developed juvenile females from ostracod (left) and snail (right) (60 diam.).
FIG. 20. Section through praesoma of juvenile within cyst in foot of snail (42 diam.).

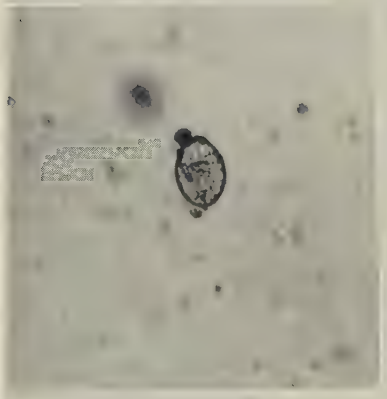
PLATE II



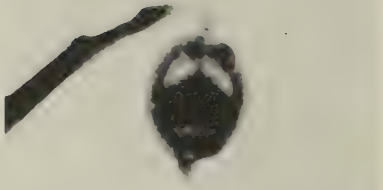
11



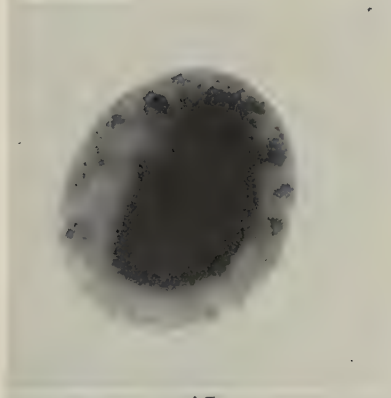
12



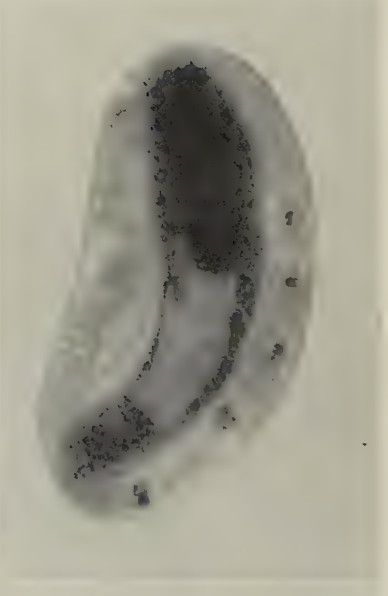
13



14



15



16

PLATE III

17

18

19

20

ABSORPTION OF PROTECTIVE ANTIBODIES FROM SERUM OF RATS IMMUNE TO THE NEMATODE, *NIPPOSTRONGYLUS MURIS*

RALPH E. THORSON

Department of Pathology and Parasitology, School of Veterinary Medicine
Alabama Polytechnic Institute, Auburn, Alabama

Sarles (1938) observed that precipitates formed at the oral and anal openings and the excretory pore of larvae of *Nippostrongylus muris* when the worms were placed in serum from rats that had been repeatedly infected with this worm. He theorized that in infections the secretions and excretions of these worms serve as antigens which stimulate formation of protective antibodies. Thorson (1953) showed by precipitin tests that antibodies to extracts of tissues of the worms and to preparations of their secretions and excretions were present in serum from rats repeatedly infected with *N. muris*. Since precipitin tests could be performed with this serum and these two types of antigens, it was felt that absorption of the antibodies to these antigens would aid in determining which of them served to stimulate the formation of protective antibodies in the infected animal.

MATERIALS AND METHODS

In general, the materals and methods used in this work are the same as described in previous papers (Thorson, 1953 and 1954). Immune serum for this experiment was obtained from four rats which had been infected with 1200, 4600, and 8100 infective larvae of *N. muris* at 6 day intervals. The blood was drawn 14 days after the last infection. The serum was separated and stored as described by Kabat and Mayer (1948). Infective larvae of *N. muris* were separated from charcoal cultures with a Baermann apparatus. They were then washed three times in sterile distilled water, twice in merthiolate (1 : 10,000) and twice more with sterile distilled water. Three million larvae which had been carefully washed were then placed in 10 ml. of sterile saline with antibiotics and the mixture was shaken for 24 hours at room temperature. After this period 1,000,000 more larvae in 3 ml. of saline and antibiotics[1] were added. The purpose of the antibiotics was to hold in check the growth of bacterial contaminants. After a total of 48 hours, the larvae, almost 100 per cent of which were still alive, were removed, and the saline containing the secretions and excretions of the larvae was used as an antigen in precipitin tests with the immune serum described above. Infective larvae were loyophilized, ground-up in a mortar with pestle and extracted with saline (Thorson, 1953) and this preparation served as another antigen in precipitin tests.

In experimental infections, 4 to 5 week old normal rats were used and were necropsied 8 days after the infection was administered. They were sacrificed with chloroform, the abdomen of each rat was opened, and the entire small intestine was removed and placed in a Petri dish with 0.85 per cent saline. The intestine was carefully slit and the adult worms were removed with a curved glass rod. The worms, which could be easily seen, were counted directly.

Received for publication, September 22, 1953.

[1] Antibiotics: Crystalline penicillin G—250 Oxford Units per ml. of fluid. Streptomycin sulfate—2.5 mg. per ml. of fluid.

EXPERIMENTAL PROCEDURE AND RESULTS

A. Precipitin tests to determine the amounts of the antigens needed to absorb homologous antibodies from immune serum of rats repeatedly infected with *N. muris.*

Since it was known that either of the antigenic solutions (a saline preparation of secretions and excretions or a saline extract of ground-up lyophilized larvae) would produce a precipitate with serum from rats repeatedly infected with *N. muris,*[2] a preliminary precipitin test was performed to determine the amount of each antigen needed to absorb completely the precipitable antibodies to either of the antigens in the immune serum. Serial dilutions (1 : 1, 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32) in the amount of 0.1 ml. of one of the antigenic solutions were added to 0.1 ml. of serum in small conical centrifuge tubes. The solutions were mixed and refrigerated at 4° C. for the observation period. Throughout this procedure as sterile conditions as possible were maintained to prevent bacterial contamination from obscuring results. After 24 hours in the cold it was found that 0.1 ml. of an undiluted saline extract of worms (as described in materials and methods) and 0.1 ml. of undiluted (1 : 1) saline which had contained living larvae both produced precipitates. The tubes were centrifuged and the supernatant solutions removed. These were then tested to determine if all the precipitable antibodies were removed This was accomplished by dividing the supernatants into 2 parts; to 1 part 0.05 ml. of the antigen was added, and to the other part 0.05 ml. of the serum was added. If there were still antibodies to the antigenic solution present in the supernatant, the tube to which the antigen was added would have more precipitate. Conversely, if there were excess antigen in the supernatant, the tube to which serum (antibody) was added would have more precipitate. This procedure was continued with the precipitate being removed each time until there was no more precipitate formed when antigen was added and when the addition of serum to the same tube would produce more precipitate. This indicated that the antigen was in excess and that all the antibodies to components of that antigen had been removed from the serum. In this preliminary test it was found that 0.3 ml. (undiluted) of either antigen would guarantee an excess of that antigen in the supernatant after a 24 hour reaction period.

B. Experimental determination that the secretions and excretions in saline stimulate formation of protective antibodies in rats.

In the previous section it was determined that 0.3 ml. of either antigen, a saline extract of ground-up larvae or saline containing secretions and excretions, would remove all the precipitable antibodies to that antigen in 0.1 ml. of immune serum. It has also been shown that injecting infective larvae of *N. muris* with immune serum into a rat will reduce the number of adults recovered in the intestine of a rat at autopsy (Thorson, In Press). This experiment combined these 2 observations in an attempt to determine which of the antigenic preparations stimulate protective antibodies in infections in rats. Five 0.1 ml. samples of immune serum were each absorbed with 0.3 ml. of the undiluted secretions and excretions in saline, and 5 were absorbed with 0.3 ml. of the undiluted saline extraction of ground-up ly-

[2] This serum will be referred to as immune serum since the rats from which it was obtained were refractory to further infection with *N. muris.*

ophilized worms. Five additional 0.1 ml. samples of immune serum were diluted to the same extent (1 : 4) with saline, and 5 0.1 ml. samples of normal serum were likewise diluted with saline. After 24 hours at 4° C., the tubes showing precipitates (the 5 tubes in which immune serum was mixed with secretions and excretions in saline and the 5 tubes in which immune serum was mixed with a saline extract of ground-up worms) were centrifuged and the precipitates removed. To each of the supernatants from this centrifugation and to each of the tubes in the other 2 groups, about 450 infective larvae[3] of *N. muris* were added. Each of these preparations was injected subcutaneously into a separate 4 week old rat.

The rats were autopsied 8 days after infection. Those that had received larvae in normal serum (and had served as controls[4]) had greater numbers of adult worms in their intestines than any of the 3 other groups (Table 1). The animals injected with larvae in immune serum which had been absorbed with secretions and excretions did not have significantly fewer adult worms than the normal serum controls,

TABLE 1.—*The numbers of adults of* N. muris *in rats' intestines after infection with 450 larvae in normal serum, immune serum, immune serum absorbed with a saline extract of lyophilized larvae and immune serum absorbed with a saline preparation of secretions and excretions of larvae**

	Mean number of adults in 5 rats	SD	SE_m	p**
Larvae injected in normal serum	248.2	16.8	8.4	(0.1–0.05)
Larvae injected in immune serum absorbed with saline containing secretions and excretions	221.0	14.6	7.3	(0.01)
Larvae injected in immune serum absorbed with a saline extract of lyophilized worms	119.8	17.1	8.55	(0.2–0.1)
Larvae injected in immune serum	103.0	13.3	6.65	

* Final serum dilution 1 : 4.
** Students "t" test.

while those receiving larvae in immune serum absorbed with a saline extract of lyophilized larvae had significantly fewer adult worms than either of the previously mentioned groups and not significantly more worms than the group receiving larvae in immune serum.

These results showed that the protective capacity of the immune serum was partially removed when it had previously been absorbed with the secretions and excretions of larvae in saline. This is an indication that the antigens present in these secretions and excretions serve as the stimuli for the formation of protective antibodies in the rat against *N. muris*. The experiment also indicates that although antibodies were present in the serum to antigens in the saline extract of ground-up larvae, these antibodies were not protective in nature.

DISCUSSION

It has been shown that immune serum reduced the worm burden in rats when it was injected with the larvae of *N. muris* (Thorson, In Press) and that absorption

[3] The number of larvae was estimated by Scott's counting chamber (1928). Frequent checks by a method in which an animal was infected and the next dose set aside for counting showed that the error of this system when used carefully was about 2 per cent.

[4] In previous work it has been observed that normal serum controls such as these never differed from controls in which saline or distilled water was used as a suspending medium for infective larvae (Thorson, 1953).

of antibodies from serum with a saline extract of ground-up lyophilized larvae did not appreciably lessen the protective ability of the immune serum. However, when immune serum was absorbed with saline containing secretions and excretions of larvae, there was a decided reduction in its ability to reduce the worm burden. This gives positive evidence for Sarles' hypothesis that the secretions and excretions stimulate the formation of protective antibodies.

From the evidence at hand it is possible to say that secretions and excretions in saline serve as the stimuli for the formation of protective antibodies in rats as evidenced by prior work in which rats injected with this antigen were partially protected from a subsequent infection. These rats also possessed antibodies in their sera which precipitated the secretions and excretions when larvae were placed in the serum (Thorson, 1953). The protective antibodies in serum could be absorbed with an antigenic solution containing the secretions and excretions of infective larvae of *N. muris*. The exact function of these antibodies in creating resistance to further infection is being studied further, but it may be, as suggested in an earlier paper (Thorson, 1953), to reduce the efficiency of the parasites' enzyme systems, thereby slowing the invading larvae and interfering with their nutrition, thus making it more difficult for them to reach their final site and to develop and maintain themselves in the host.

SUMMARY

1. Antibodies were absorbed from serum of rats repeatedly infected with *Nippostrongylus muris* by a saline extract of ground-up lyophilized larvae or by saline which contained the secretions and excretions of living larvae.

2. This immune serum which was absorbed with the saline containing secretions and excretions of larvae lost part of its protective ability when it was injected with infective larvae of *N. muris* into rats.

3. Immune serum which was absorbed with a saline extract of ground-up lyophilized larvae lost none of its protective capacity when it was injected with infective larvae of *N. muris* into rats.

References

Kabat, E. A. and Mayer, M. M. 1948 Experimental Immunochemistry. First Edition. Charles C Thomas, Publisher, Springfield, Illinois.

Sarles, M. P. 1938 The in vitro action of immune rat serum on the nematode, *Nippostrongylus muris*. J. Infect. Dis. **62**: 337–348.

Thorson, R. E. 1953 Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris*. Am. J. Hyg. **58**: 1–15.

———1954 The effect of immune serum from rats on the infective larvae of *Nippostrongylus muris*. Exper. Parasit. **3**: 9–15.

A PRELIMINARY, ANNOTATED LIST OF TICKS (IXODOIDEA) OF THE ANGLO-EGYPTIAN SUDAN

HARRY HOOGSTRAAL

Head, Department of Medical Zoology
U. S. Naval Medical Research Unit No. 3
Cairo, Egypt

The following briefly annotated list of tick species of the Anglo-Egyptian Sudan is presented for its timeliness in view of the lively interest that has developed in African ticks during the last few years. These data are extracted from a monographic work now in progress with special reference to the Anglo-Egyptian Sudan, concerning African tick-borne diseases and the ticks which are incriminated with them.

The tremendous land area covered by the Anglo-Egyptian Sudan includes tropical forest, various types of forested and unforested grasslands, swamps, semi-desert, and extreme desert. A large proportion of the ticks of tropical Africa occur in the southern Province (Equatoria) or Provinces, but are totally absent in the central Provinces. Few of the species confined to the southern and western parts of the Anglo-Egyptian Sudan have heretofore been collected in this country, and many records included here represent a considerable extension of known ranges. Among the large numbers of specimens studied from a wide variety of hosts, only a single undescribed species has been recognized.

King (1926) published the last survey of Anglo-Egyptian Sudan ticks, in which he listed 22 species. One of these, *Hyalomma aegyptium,* we now know does not occur in the Sudan, although several other species in this genus are common there. His specimens identified as *Rhipicephalus falcatus* consist of four different species. Since 1926, 1 other species has been reported for the Sudan and a few brief notes on ticks already known to be present have been published.

In the present list are included 60 species plus probably 2 additional subspecies of *Haemaphysalis leachii* and 1 additional subspecies of *Rhipicephlus simus.* Old records of species erroneously attributed to the Sudan are disregarded here but are discussed in the larger work in progress.

The materials on which these records are based are the result of a 3 year study of some 75,000 specimens from the following sources: (1) Sudan Veterinary Service, through the kindness of the Director, Mr. J. T. R. Evans; (2) Sudan Government Collection at Wad Medani, through the kindness of Mr. D. J. Lewis, Medical Entomologist; and (3) my collection made on several trips from 1948 to this year. Specimens in the British Museum (Natural History) were also studied through the kindness of Mr. E. Browning and Dr. G. Owen Evans.

Equatoria Province records are based almost entirely on material collected by myself, with the assistance of several friends, and number more than 25,000 specimens. I have collected to a lesser extent in the central and northern Provinces.

Received for publication, December 21, 1953.

The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Full acknowledgment to many friends in the Sudan and abroad who have contributed to this project in one way or another will appear in the completed work.

In the following list, Province names in which each species is known to occur are capitalized. If the record has not previously been published, an asterisk (*) follows the Province name. A map, Figure 1, with Province names marked, is included for reference.

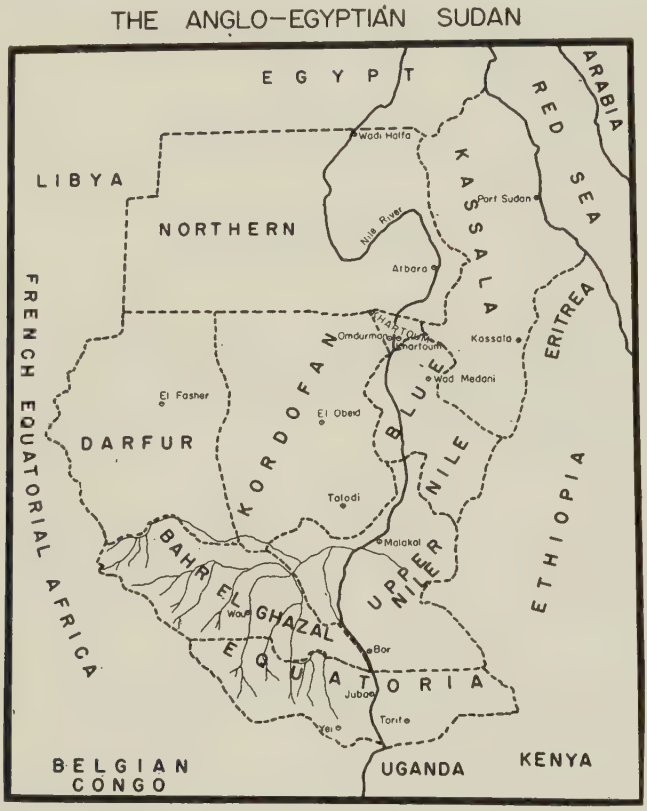


FIG. 1. Map of the Anglo-Egyptian Sudan, showing provinces, rivers, and some cities.

ARGASIDAE

ARGAS

A. brumpti Neumann, 1907.

EQUATORIA*, BLUE NILE, KASSALA, KORDOFAN. Larvae from eastern Equatoria in association with hyraxes and spiny mice (*Acomys*).

A. boueti Roubaud and Colas-Belcour, 1933.

EQUATORIA*. Larvae and nymphs from *Taphozous perforatus haedinus.*

A. persicus (Oken, 1818).

All provinces. Common on domestic chickens.

A. reflexus (Fabricius, 1794).

EQUATORIA*. Apparently rare. Not otherwise known from the Ethiopian Faunal Region except in French West Africa.

A. sp. near *fischeri* Audouin, 1827.

NORTHERN*, KHARTOUM*. Only larvae and nymphs available.

A. vespertilionis (Latreille, 1796) (group).
EQUATORIA*, KHARTOUM, NORTHERN. Only larvae and nymphs available.

ORNITHODOROS

O. moubata (Murray, 1877).
EQUATORIA, BAHR EL GHAZAL*. The Bahr el Ghazal specimens were taken from warthog burrows near Guar in Gogrial Subdistrict by E. T. M. Reid and P. Blasdale. Equatoria specimens are from human habitations.

O. savignyi (Audouin, 1827).
BLUE NILE, KHARTOUM, KASSALA, KORDOFAN, DARFUR, NORTHERN. Common in camel yards and domestic animal resting places.

IXODIDAE

AMBLYOMMA

A. cohaerens Dönitz, 1909.
EQUATORIA*. Adults on buffalos only in far eastern part of Province. Nymphs, tentatively determined as this species by Dr. G. Theiler, from tree rat (*Thamnomys*) and three species of ground-feeding birds.

A. lepidum Dönitz, 1909.
EQUATORIA, BAHR EL GHAZAL, UPPER NILE, KASSALA, DARFUR*, KORDOFAN. Transient but not established in Northern Province; probably not established in Bahr el Ghazal and western half of Equatoria. Commonly attacks all domestic animals, also antelopes and forest buffalos; frequent on camels in central provinces.

A. marmoreum Koch, 1844 (group).
EQUATORIA*, KHARTOUM. Khartoum specimens are probably from imported tortoises. On leguan lizards and tortoises in Equatoria.

A. nuttalli Dönitz, 1909.
EQUATORIA*, KORDOFAN. *A. werneri werneri* Schulze, 1932, from Kordofan, is a synonym of *A. nuttalli*. Adults from tortoises and *Varanus* lizards, nymphs from oribi antelope and spur-fowl (*Francolinus*).

A. pomposum Dönitz, 1909.
EQUATORIA*. A single specimen from a cow at Yei.

A. rhinocerotis (de Geer, 1778) (= *A. petersi* Karsch, 1878).
EQUATORIA, UPPER NILE. Rare.

A. tholloni Neumann, 1899.
EQUATORIA. Large numbers of adults (only) from elephants; a single nymph, identified by Dr. G. Theiler, from a chameleon. Not found on elephants which have been examined in other Provinces.

A. variegatum (Fabricius, 1794).
EQUATORIA, BAHR EL GHAZAL, BLUE NILE*, UPPER NILE, KORDOFAN, DARFUR*. Rare in Blue Nile, uncommon in Kordofan and Darfur, transient but not established in Khartoum and Northern; very common most everywhere else. Usual cattle parasite of Sudan; also common on antelopes and buffalos; relatively few on smaller domestic animals.

APONOMMA

A. exornatum (Koch, 1844).
EQUATORIA, BLUE NILE*, UPPER NILE*, KORDOFAN*, KASSALA*, ? KHARTOUM. The Khartoum specimens may have come from lizards in the zoological gardens, Found only on *Varanus* lizards.

A. latum (Koch, 1844) (= *A. laeve capensis* Neumann, 1901, of authors).
EQUATORIA, UPPER NILE, BAHR EL GHAZAL*. From large, poisonous snakes only; none found on many small snakes examined.

BOOPHILUS

B. annulatus (? *congolensis* Minning, 1934).
EQUATORIA*, BAHR EL GHAZAL*, UPPER NILE*, KORDOFAN*. Rare in Kordofan, transient but not established in Northern Province; fairly common in the South. I cannot differentiate African specimens from American *B. annulatus*. Sudan hosts are usually cattle, once an eland and once a donkey.

B. decoloratus (Koch, 1844).
EQUATORIA, BAHR EL GHAZAL, UPPER NILE, ? BLUE NILE, KORDOFAN, DARFUR, KASSALA. Does not reach northern Kordofan and Darfur; rare in humid coastal areas and hills of Kassala. Rare on antelopes; common on cattle, uncommon on other domestic animals except locally on dogs which sleep in huts with cattle.

DERMACENTOR

D. circumguttatus Neumann, 1897.
EQUATORIA*. Specimens from grass near Kajo Kaji, rare.

D. rhinocerinus Denny, 1843 (= *D. rhinocerotis* of authors).
EQUATORIA. Rare.

HAEMAPHYSALIS

H. aciculifer Warburton, 1913.
EQUATORIA, BAHR EL GHAZAL*. Immature specimens from rodents and oribi antelope, adults from large antelopes and cattle; not common.

H. sp. nov.
EQUATORIA*. A hyrax parasite from both the eastern and western parts of the Province; related to the *H. hoodi* group of Africa and Madagascar and to the *H. doenitzi* group of Southern Asia and Palawan (Philippines).

H. hoodi hoodi Warburton and Nuttall, 1909.
EQUATORIA*, BAHR EL GHAZAL*. Specimens from spur-fowl (*Francolinus*), Abdim's stork, and tchagra shrike.

H. houyi Nuttall and Warburton, 1915. (new combination).
EQUATORIA*, BAHR EL GHAZAL*, UPPER NILE*, BLUE NILE. All specimens from ground squirrels. Comparison of Sudan specimens with others from West Africa and with typical *H. calcarata* from Somaliland and Kenya indicates that *H. houyi* is a distinct species and not a subspecies of *H. calcarata*, as previously considered. Neumann's (1910) description of the female of *H. calcarata*, from Roseires, Blue Nile, apparently refers to *H. houyi* and not to *H. calcarata*.

H. leachii (Audouin, 1827) subspp.
All Provinces. I am at present engaged in a study of this African-Asian complex which will take months to complete. Sudan specimens appear to include typical *H. leachii leachii, H. leachii humerosoides* Theiler, 1943, and *H. leachii* "subspecies near *indica*".

H. erinacei Pavasi, 1884 (= *H. numidiana numidiana* Neumann, 1905).
EQUATORIA*. A pair of ticks collected from a hedgehog at Juba; the only record of this species from the Ethiopian Faunal Region except for one questionable record from the Union of South Africa.

H. parmata Neumann, 1905.
EQUATORIA*. Mountains in the eastern part of the Province. Larvae and nymphs from forest guinea fowl, adults from blue duiker and cattle.

HYALOMMA

Species names used here are those proposed by Delpy (1949A, B).

H. brumpti Delpy, 1946.
KASSALA*, KORDOFAN*, DARFUR*. Domestic animals.

H. detritum Schulze, 1919.
KASSALA*, KORDOFAN*. Domestic animals.

H. dromedarii Koch, 1844.
BLUE NILE*, KHARTOUM*, KORDOFAN*, KASSALA*, DARFUR*. Mostly camels; not infrequently cattle; rarely other domestic animals.

H. excavatum Koch, 1844.
KHARTOUM*, BLUE NILE*, KORDOFAN*, KASSALA*. Domestic animals, especially horses.

H. impressum Koch, 1844.
DARFUR*, KORDOFAN*. Domestic animals.

H. rufipes rufipes Koch, 1844.
All Provinces*. Scattered infestations on cattle; common on giraffes and buffalos in the South.

H. savignyi (Gervais, 1844).
KORDOFAN*. Domestic animals.

H. transiens Delpy, 1949.
EQUATORIA*, BAHR EL GHAZAL*, UPPER NILE*, BLUE NILE*, KORDOFAN*, DARFUR*. Frequently in few numbers on cattle and all other domestic animals; in larger numbers on buffalos and giraffes; also infests antelopes, wild pigs, warthogs, and tortoises.

IXODES

I. cavipalpus Nuttall and Warburton, 1908.
EQUATORIA*. Adults from a civet in the eastern part of the Province.

I. nairobiensis Nuttall, 1916.
EQUATORIA*, UPPER NILE*. Nymphs from mice in the plains and forested highlands of the eastern part of Equatoria, a female from a dog in Upper Nile Province.

I. rasus Neumann, 1899, ? subspecies.
EQUATORIA*. Sudan material consists of a single male from a hare at Torit. This specimen does not correspond with described subspecies. Material reported as this species by King (1726) is unidentifiable or not this species.

I. schillingsi Neumann, 1901.
EQUATORIA*. From Colobus monkeys in forested mountains in eastern Equatorial Province.

I. simplex Neumann, 1906.
EQUATORIA*. From *Rhinolophus clivosus zambesiensis* in mountains of eastern Equatoria Province.

I. vespertilionis Koch, 1844.
EQUATORIA*. Nymphs from various insectivorous bats in central parts of east bank of Equatoria.

RHIPICEPHALUS

R. appendiculatus Neumann, 1901.
EQUATORIA*. From cattle, sheep, goats, man, and a roan antelope; common just west of Nile from Yei to the Uganda border, where East Coast Fever also occurs; rare in eastern Equatoria Province.

R. arnoldi Theiler and Zumpt, 1950.
EQUATORIA*. The only record of this species except for South Africa. These specimens, identified by Dr. G. Theiler, consist of a larva from a rodent and adults from a hare in eastern Equatoria Province.

R. bequaerti Zumpt, 1950.
EQUATORIA*. One collection, identified by Dr. G. Theiler, from goats in the mountains of the eastern half of the Province.

R. compositus Neumann, 1897 (= *R. ayeri* Lewis, 1933).
EQUATORIA*. Specimens from buffalos on both banks of the Nile. The type locality of *R. compositus* is Khartoum. This must refer either to "somewhere in the Sudan" or, less likely, to specimens brought to Khartoum on animals imported from the Southern Sudan.

R. cuspidatus Neumann, 1906.
BAHR EL GHAZAL*. This is not the *R. cuspidatus* of Zumpt (1950), an entirely different species the true identity of which I do not know. Common on most warthogs in the Galual-Nyang Forest, Gogrial Subdistrict.

R. evertsi evertsi Neumann, 1897.
All Provinces, decreasing in frequency from south to north. Chiefly on domestic animals, also on antelopes.

R. köchi Dönitz, 1905.
EQUATORIA*. Rare on cattle in mountains in the eastern half of Equatoria Province.

R. longicoxatus Neumann, 1905.
BAHR EL GHAZAL*. From buffalo, identity confirmed by Dr. G. Theiler.

R. longus Neumann, 1907 (new combination).
EQUATORIA*. Specimens from buffalos, not common.

R. lunulatus Neumann, 1907.
BAHR EL GHAZAL*. Horses and antelopes, not common.

R. mühlensi Zumpt, 1943.
EQUATORIA*. Nymphs from hyraxes, adults from cattle; occurs both east and west of the Nile. Sudan specimens were compared with type material by Dr. G. Theiler.

R. pravus Dönitz, 1910.
EQUATORIA*. Common especially on domestic animals and hares in the more eastern parts of Equatoria, gradually decreasing in numbers towards the Nile, absent west of the Nile. Both sexes feed on man.

R. sanguineus sanguineus (Latreille, 1806).
All Provinces. This material has not yet been studied for the possible presence of *R. secundus* Feldman-Muhsam, 1952. Common on hares and on domestic goats and dogs; taken feeding on man; seldom on cattle; unusual on other mammals but sometimes on large ground birds.

R. simpsoni Nuttall, 1910.
EQUATORIA*. On cane-rats in both eastern and western Equatoria Province.

R. simus senegalensis Koch, 1844.
EQUATORIA*, BAHR EL GHAZAL*. Throughout these Provinces on wild hunting dogs (*Lycaon*), buffalos, warthogs, elephants, and domestic dogs. Some specimens from elephants intergrade with *R. simus simus*.

R. simus simus Koch, 1844.
All Provinces. Very common in the south, gradually decreasing in numbers towards the north. Several records of both sexes feeding on man. Immatures on several kinds of rodents; adults on hares, carnivores, and buffalos; also less commonly on antelopes, hippopotamus, warthogs, and bushpigs. Common on domestic dogs, cattle, and pigs.

R. sulcatus Neumann, 1908.
BAHR EL GHAZAL*. Few specimens from oribi antelope, leopard, and man. Specimens reported by King (1926) as this species from the Sudan have all proven to be different species on subsequent study.

R. supertritus Neumann, 1907.
EQUATORIA*. Rare on buffalos near the west bank of the Nile.

SUMMARY

An annotated list is provided of 60 species (plus probably three additional subspecies) of ticks now known to occur in the Anglo-Egyptian Sudan. Previously, 23 species had been reported from this area.

The following new name combinations are used: *Boophilus annulatus* (*? congolensis*), *Haemaphysalis houyi*, and *Rhipicephalus longus*.

Amblyomma werneri werneri Schulze, 1932, is considered a synonym of *A. nuttalli* Dönitz, 1909. The original female description of *Haemaphysalis calcarata* is believed to actually refer to *H. houyi*.

REFERENCES

DELPY, L. P. 1949A Révision par des voies expérimentales du genre *Hyalomma* C. L. Koch 1844 (2e partie). Ann. Parasit. **24**: 97–109.

————1949B Essai critique de synonymie du genre *Hyalomma* C. L. Koch 1844 depuis Linné, 1758. Ann. Parasit. **24**: 464–494.

KING, H. H. 1926 The ticks (Ixodoidea) of the Sudan. Bull. 23, Wellcome Trop. Research Lab., Entom. Sect., 21 pp.

NEUMANN, L. G. 1910 Sur quelques espèces d'Ixodidae nouvelles ou insuffisamment connues. An. sci. nat. (b) zool. 9s., **12**: 161–176.

ZUMPT, F. 1950 Preliminary study to a revision of the genus *Rhipicephalus* Koch. Document. Moçambique. **60**: 57–125 (1949).

PAURORHYNCHUS HIODONTIS, A NEW GENUS AND SPECIES OF TREMATODA (BUCEPHALIDAE: PAURORHYNCHINAE N. SUBFAM.) FROM THE MOONEYE FISH, *HIODON TERGISUS*[1]

E. Eugene Dickerman

Department of Biology, Bowling Green State University, Bowling Green, Ohio

Mooneye fish, *Hiodon tergisus*, seined from the Maumee river at Grand Rapids, Ohio, late in the summer of 1944 harbored in their body cavities a new trematode of the family Bucephalidae. In subsequent years, 300 of the new parasites have been obtained from 35 fish 150 cm. in length or longer; fish less than 150 cm. long were all negative. The number of parasites per fish varied from one to 36. Several distinctive morphological characteristics and the habitat of this worm make it impossible to assign it to any of the existing genera of the family. The author, therefore, erects the new genus *Paurorhynchus* with *Paurorhynchus hiodontis* n. sp. as type species. It is also proposed that *Paurorhynchus hiodontis* be placed in a new subfamily, Paurorhynchinae, since it is impossible to assign the new genus to either of the two subfamilies erected by Nicoll (1914).

Paurorhynchus hiodontis n. g., n. sp.
(Figs. 1, 4)

Diagnosis: Bucephalidae: Paurorhynchinae, n. subfam.: Body elongate, nearly circular in cross-section, averaging 6.38 mm. (8.0–5.25) long by 1.68 mm. (2.06–1.37) wide, ends tapering in living condition but rounded in flattened and preserved specimens. Cuticula thin, without spines. Anterior end in living specimens extended in form of a small rhynchus; rhynchus retracted in fixed specimens causing anterior end to appear to bear a weak sucker. Mouth midventral, about one-third length of body from anterior end; prepharynx very short; pharynx averaging 0.168 mm. (0.21–0.133) in diameter; esophagus short, directed anterodorsally, expanding to form an elongated digestive sac which turns and extends posteriorly to region of anterior testis. Excretory bladder long and tubular, extending from near pharynx to near posterior end of body, and terminating with a short excretory duct leading to the terminal pore; main excretory canals opening into excretory bladder near posterior end. Testes in posterior two-fifths of body, oblique, much lobed, anterior testis slightly larger and more lobed than posterior. Cirrus pouch, situated in mid-line of body near posterior end, half as long as posterior testis, somewhat curved, moderately thick walled; seminal vesicle internal, pars prostatica short, surrounded by prostate gland cells; genital aperture terminal, posterior. Ovary much smaller than testes, variable in shape and number of lobes, situated on right side of body at level of anterior testis or slightly posterior to it. Oviduct coursing posteriorly from ovary, giving off Laurer's canal and receiving common vitelline duct. Oötype posterior to ovary. Uterus at beginning narrow and looping but gradually broadening and straightening in anterior end of body, bending sharply it then courses posteriorly to terminate in the metraterm opening into genital sinus; uterus in gravid specimens occupying all available space. Vitellaria consisting of 12 to 17 small follicular masses in linear series, loosely separated on each side of body, and usually extending from a level midway between mouth and anterior end of body to level of anterior testis. Vitelline ducts extending posteriorly from series of follicles to level of ovary where left one crosses the body to join right duct in vicinity of ovary. Discharged eggs (Fig. 2) elongated oval with prominent operculum and abopercular knob and average 0.072 mm. (0.08–0.06) long by 0.032 mm. (0.04–0.028) wide.

Development: Unknown.

Host: Hiodon tergisus.

Received for publication, October 23, 1953.

[1] Contribution from the Franz Theodore Stone Institute of Hydrobiology of the Ohio State University, Put-in-Bay, Ohio and the Biology Department of Bowling Green State University, Bowling Green, Ohio.

Location: Mature specimens in body cavity; immature specimens in liver.
Locality: Lake Erie and southern tributaries.
Type: Holotype and paratype in the Helminthological Collection, U. S. National Museum, No. 37404.

Paurorhynchus n. g.

Diagnosis: BUCEPHALIDAE: PAURORHYNCHINAE, n. subfam.: Body elongate, cylindrical. Cuticula thin, without spines. Anterior end with weak inconspicuous rhynchus only. Oral aperture ventral, without sucker, pharynx present, caecum an elongated arched sac. Excretory bladder elongate. Testes oblique, much lobed, in posterior two-fifths of body. Cirrus pouch half as long as posterior testis. Genital aperture terminal at posterior end of body. Ovary lobed, much smaller than testes, situated on right side of body on level with anterior testis. Seminal receptacle absent. Laurer's canal present. Vitellaria small follicular masses in linear series on sides of body. Mature adults in body cavity of host.
Type species: Paurorhynchus hiodontis.

Paurorhynchinae n. subfam.

Diagnosis: BUCEPHALIDAE; Body elongate. Cuticula thin, without spines. Anterior end with weak, inconspicuous rhynchus only. Ovary and testes lobed. Ovary dextral, testes oblique. Mature adults in body cavity of fishes.
Type and only Genus: Paurorhynchus.

MIRACIDIUM

The following description of the miracidium of *P. hiodontis* (Fig. 3) is based upon two embryos which appeared in the uterus of a *toto* mount and upon many specimens which were forced from discharged eggs with gentle pressure. The miracidium is pyriform, the anterior end being sharply acute, the posterior end bluntly obtuse. The over-all length from tip of stylet to posterior end of appendages is 0.1 mm.; the greatest width is 0.035 mm. near the posterior end. The spindle shaped stylet measures 0.018 mm. in length by 0.003 mm. in diameter and is enclosed within the pointed anterior portion of the body to which are attached the five narrow and elongated ciliated plates by their anterior ends. The plates curve slightly with the contour of the body and end just short of its posterior tip. The position of the four paired appendages in respect to dorsal ventral and lateral regions of the embryo could not be determined since the miracidium was never seen in motion. Each appendage is very slender, unjointed, bearing many long cilia at its posterior end. Cilia are present on the cephalic plates and appendages only. Flame cells were not observed. The miracidium of *P. hiodontis* differs from that of *Bucephalus papillosus* Woodhead, 1929, and of *B. elegans* Woodhead, 1930, in possessing five cephalic plates instead of four, and two pairs of unjointed appendages instead of three pairs of jointed ones.

DISCUSSION

Although *P. hiodontis* differs from described species of the BUCEPHALIDAE in being of large size, lacking cuticular spines, having lobed gonads and living in the body cavity of the host, its general morphology and the structures of the miracidium require that the species be allocated to this family. Specimens of *P. hiodontis* were first found during July and August and at that time of the year the worms contained few or no eggs and their development had not proceeded beyond the 4-cell stage. Subsequent attempts were made to collect fish at other times of the year so that miracidia could be observed. However, fish from the Maumee river were not obtainable except in late July and August. It was possible, however, to obtain living

Mooneyes from Lake Erie in late March and early April of 1953 through the cooperation of Mr. C. F. Nielsen, a commercial fisherman, at Venice, Ohio where he has facilities for keeping fish alive. From these fish taken during March and April, 50 specimens of *P. hiodontis* were obtained and each worm was heavily laden with eggs containing miracidia.

It was assumed that the parasite did not occur in the Mooneyes in Lake Erie because Bangham and Hunter (1939) in examining 28 of these fish from this source did not find it. In a conversation with Bangham it was learned that they had obtained their fish from commercial fishermen and that an hour or more had elapsed from the time the fish were taken from the water and their examination. Since the large Mooneyes which were being taken from the Maumee river were 100 per cent infected it was easy to discover that the parasites die soon after the death of the host and have deteriorated so badly by the end of an hour that it is impossible to detect them.

Paurorhynchus hiodontis is larger than any trematode of the family BUCEPHALIDAE yet described, flattened and fixed specimens averaging more than 6 mm. in length. Living specimens in 0.6 per cent sodium chloride were seen to extend to more than double that length. A few specimens kept in saline at room temperature in August were still active at the end of four days. This is in sharp contrast, as stated above, to the rapid deterioration of the parasites permitted to remain in the body cavity of the host after its death. The parasite is a very light pinkish color when alive and with few eggs in the uterus; the color is more olivaceous in gravid specimens collected in April. However, when gravid worms are placed in water, thousands of eggs are rapidly discharged and the pinkish color returns. It is assumed, since Mooneyes have abdominal pores, that the parasites are dispersed from the host at the time of spawning and that the eggs are discharged when the parasites come into contact with the water.

Numerous discharged eggs were kept in lake water in both sealed and unsealed small stender dishes and observed periodically from early April until early June, 1953. During that period not a single miracidium hatched. In only a few instances were cilia seen to move, and then for a short time only, when the miracidium was forced from the shell with gentle pressure upon the cover glass. In no instance were cilia observed in movement on miracidia within the shells. Later, when studying the prepared *toto* mounts of specimens which had shed eggs, many eggs remaining within their uteri were observed with the operculum ajar and with the miracidium partially protruding from the opening. In one *toto* mount 2 empty shells were observed and the intact miracidia were near by. All specimens for such mounts had been flattened under gentle pressure and killed with Bouin's fixative at room temperature. It seems possible, therefore, that stimulation by acid may be necessary for the proper hatching of the eggs of this worm. Further study of the miracidium is planned for the spring of 1954 when it is hoped that a more detailed description of that stage can be obtained.

In addition to the history of the systematics of the family Bucephalidae as reviewed by Kniskern (1952), attention should be called to two important papers (Manter, 1934 and Manter, 1940, containing descriptions of 13 new species, representing five genera of bucephalids).

SUMMARY

1. A new genus and species of gasterostome, *Paurorhynchus hiodontis* is described from the body cavity of the Mooneye fish, *Hiodon tergisus,* and placed in the newly created subfamily, PAURORHYNCHINAE, in the family BUCEPHALIDAE.

2. The new trematode differs from all other described species of bucephalids in being considerably larger, in being spineless, in having lobed gonads and by living in the body cavity of the host.

3. The miracidium is of the bucephalid type, but differs in having five ciliated plates instead of four and two pairs of unjointed appendages instead of three pairs of jointed ones.

LITERATURE CITED

BANGHAM, R. V. AND HUNTER, G. W., III 1939 Studies on fish parasites of Lake Erie. Distribution Studies. Zoologica, New York Zool. Soc. **24** (4) : 385–448.

KNISKERN, V. B. 1952 Studies on the trematode family Bucephalidae Poche, 1907. Part I. A systematic review of the family Bucephalidae. Trans. Amer. Micr. Soc. **71** (3) : 253–266.

MANTER, H. W. 1934 Some digenetic trematodes from deep-water fish of Tortugas, Florida. Pub. (435), Carnegie Inst. Wash., Papers Tortugas Lab. **28:** 257–345.

———1940 Gasterostomes (Trematoda) of Tortugas, Florida. Pub. (524), Carnegie Inst. Wash., Papers Tortugas Lab. **33:** 1–19.

NICOLL, W. 1914 The trematode parasites of fishes from the English Channel. J. Marine Biol. Ass. United King. **10** (3) : 466–505.

WOODHEAD, A. E. 1929 Life history studies on the trematode family Bucephalidae. Trans. Amer. Micr. Soc. **48** (3) : 256–275.

———1930 Life history studies on the trematode family Bucephalidae. No. II. Trans. Amer. Micr. Soc. **49** (1) : 1–17.

EXPLANATION OF PLATE

All drawings pertain to *Paurorhynchus hiodontis* n. g., n. sp. and were made with the aid of a camera lucida.

Abbreviations used: A, appendage; CP, ciliated plate; EB, excretory bladder; LC, Laurer's canal; MG, Mehlis' gland; O, ovary; P, pharynx; R, rhynchus; RG, rhabdocoel gut; S, stylet; T, testis; U, uterus; V, vitellaria.

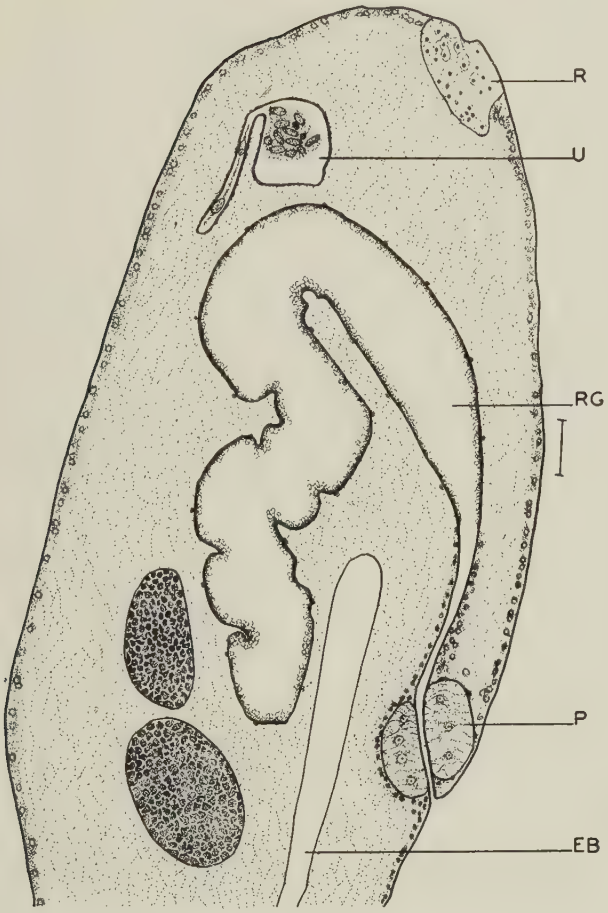
FIG. 1. Mid-sagittal section of an unflattened specimen. Scale, 0.1 mm.

FIG. 2. Mature egg. Scale, 0.01 mm.

FIG. 3. Miracidium. Scale, 0.01 mm.

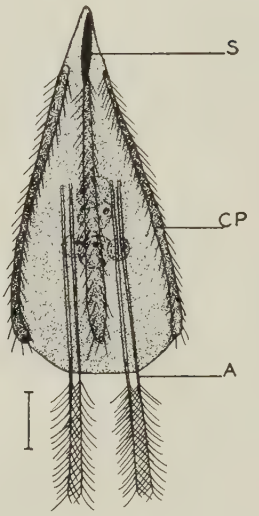
FIG. 4. Mature flattened specimen. Scale, 1.0 mm.

PLATE I

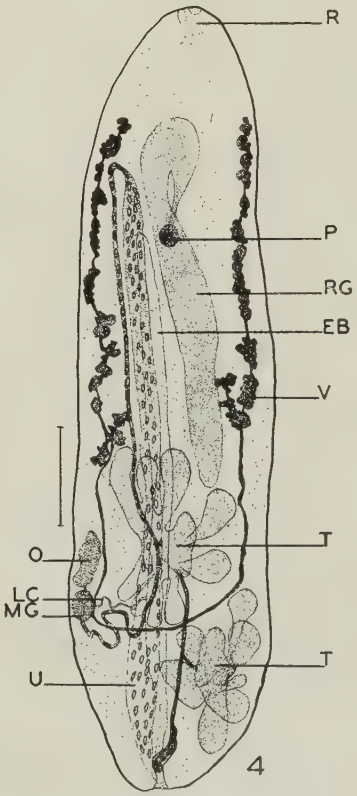


1

2



3



4

EVIDENCE OF TISSUE INVASION IN CHICKS BY *EUHAPLORCHIS CALIFORNIENSIS* MARTIN, 1950, (TREMATODA: HETEROPHYIDAE)[1]

Lois W. Wong[2]

Department of Zoology, University of Southern California

INTRODUCTION

The present paper deals with the degree of intramucosal invasion of *Euhaplorchis californiensis* in chicks, the location of the flukes in the intestine, and the effect of the continuous feeding of large numbers of these flukes to chicks.

Euhaplorchis californiensis Martin, 1950, is a small trematode of the family Heterophyidae. This family is widely distributed and many of its members can parasitize man. Africa (1937, 1938), Africa, Garcia and de Leon (1935), and Africa, de Leon and Garcia (1935, 1936a, b, c, d, 1937 and 1940) reported that some heterophyid species invaded the intestinal tissue of man and other animals. With the death and disintegration of the flukes, the ova were picked up by the lymph or blood and carried to various parts of the body with serious results.

In humans the visceral complications were found to involve heart, brain, spinal cord, liver, lung and spleen. In cattle egrets parasites were found in the portal area of the liver. In sea gulls eggs were found in the pancreas. Africa (1937) fed *Haplorchis yokogawai* to various hosts such as kittens, pups, and birds (cattle egret, pelican, and owl) and found tissue invasion only in dogs and cattle egret. Africa considered the failure of detecting intramucosal invasion in the kitten to be due to light infections. According to him, once these trematodes were inside the tissue, they did not return to the lumen to complete their reproductive activity as suggested by previous authors, but rather became incarcerated and remained there until death.

Yokogawa (1913) found that young *Metagonimus yokogawai* invaded the intestinal tissues of dogs and mice. Ciurea (1924) found a young *Metagonimus romanicus* in a gland of Lieberkühn. He concluded from this observation that all heterophyid worms were characterized by an intramucosal stage of development. Faust and Nishigori (1926) also suggested the invasion of host tissue by *Monorchotrema* (synonym—*Haplorchis,* Looss, 1899) *taihokui* and *M. taichui.* However, Stunkard and Willey (1929) in their experimental work on the development of *Cryptocotyle lingua* in cats and rats found no evidence of tissue invasion. Negative results were found by Willey and Stunkard (1942) in terns and dogs. They suggested that members of certain heterophyid species invade the tissue of some hosts. When invasion occurs, it is accomplished by small, recently excysted worms.

Received for publication, November 2, 1953.

[1] Part of a dissertation submitted to the University of Southern California in partial fulfillment of requirements for the degree of Doctor of Philosophy. Appreciation is expressed to Professor W. E. Martin, University of Southern California, for his valuable suggestions and advice throughout this study.

[2] Now at the Department of Parasitology, School of Tropical and Preventive Medicine, Loma Linda, California.

MATERIALS AND METHODS

New Hampshire chicks kept at 32° to 34° C. were used. The chicks were given no food or metacercariae until they were at least 24 hours old, and the initial feeding of parasites occurred before the birds were 2 weeks old. They were given food for some time before being fed with brains of *Fundulus parvipinnis parvipinnis,* infected with the metacercariae of *Euhaplorchis californiensis.* The fish were collected in a brackish canal at Playa del Rey, California.

Since the writer had previously found that the intensity of infection by *E. californiensis* could be increased through feeding the chicks with oatmeal and fasting them after they had ingested the metacercariae, this method was used to facilitate tissue invasion that might occur during the course of development of the worms in the intestine. The chicks were fed oatmeal for about 10 days after hatching and then given an equal mixture of oatmeal and chick feed in order to prolong their lives, since they usually die in about two weeks if fed oatmeal alone. Each of a group of 26 chicks was given a single feeding of 20 infected fish brains during its first two weeks of life. This amounted to approximately 9,000 metacercariae per chick. Two chicks of this group were killed at intervals of 5, 10, and 15 hours, and also at intervals of 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 days after receiving fish brains. The purpose of the experiment was to infect the chicks as heavily as possible in order to determine whether or not tissue invasion would occur.

Since it was found that the majority of *E. californiensis* are located in the last 4 inches (approximately 10 cm.) of the small intestine, a 10 mm. segment at the end of the small intestine was opened and the flukes inside counted. A segment of intestine 30 mm. long immediately in front of the first segment was excised, fixed in hot Bouin's fluid and serially sectioned for histological study. Some of the sections were stained with iron hematoxylin and destained with picric acid; others were stained with Delafield's hematoxylin and counterstained with triosin.

In another group, 6 chicks were fed daily 4 to 25 infected fish brains each, depending upon the number of fish available. The number of metacercariae present on each fish brain varied from 200 to more than 1,000, the average being approximately 450 per brain. Five of these infected chicks were fed with oatmeal during their first 10 days of life, and the other bird was fed chick feed. After the chicks were about 10 days old, those receiving oatmeal were fed oatmeal mixed with chick feed. Since the bird fed only chick feed died on the eighth day, 1 of the other chicks fed oatmeal was killed and examined on the 8th day so that the degree of infection in the 2 hosts could be compared. Of the remaining 4 chicks, 1 died on the 10th day, another on the 16th and the other 2 were sacrificed on the 43rd day. The last 2 were fed a total of 516 and 484 fish brains respectively. These birds were fed fish brains almost daily for 36 days and were killed 7 days after the last infective feeding. The last 10 mm. of the small intestines of all these chicks were examined, and the 30 mm. segments of small intestines immediately anterior to the last 10 mm. of intestine were fixed for histological examination.

RESULTS AND DISCUSSION

In chicks sacrificed 5 hours after infection, an average of only 1 fluke was found in the last 10 mm. segment of the small intestine, although numerous worms were recovered in the portion of the intestine immediately anterior to that segment.

Chicks sacrificed during the first 3 days after infection had an average of more than 100 flukes in the last 10 mm. segment of the small intestine. The number of parasites in this area decreased after the first 3 days, none being found here after 15 days or in the entire intestine after 30 days.

The serial sections of chick intestines showed that most of the flukes (67%) were between the villi. There were areas of intestine in which nearly every space between the villi was occupied by one or more worms. Approximately 15% of the parasites were found either partially or entirely within the lumen of the intestinal glands and only rarely were 2 flukes found in the lumen of a single gland. About 13.5% were found in the lumen of the intestine and 3% pressed against the muscularis mucosae. No trematodes were found in the muscularis. A small number (1.5%) was imbedded in the tunica propria (See Plate I). Only worms 10 hours to 3 days of age (majority about 15 hours old) were found to invade the tissues. One 3-day-old fluke with eggs was found within the tunica propria.

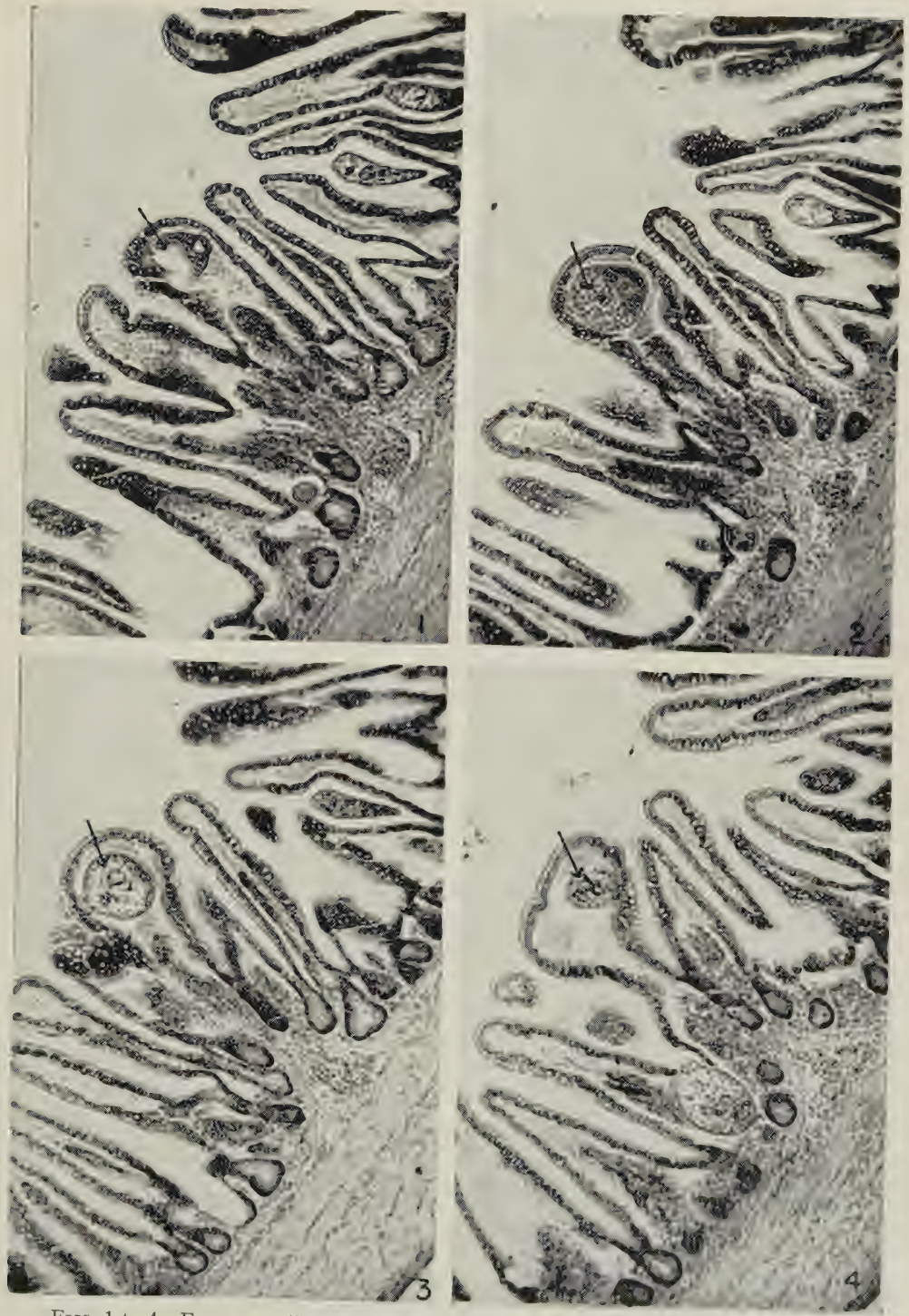
The worms were found most frequently with their oral suckers directed toward the muscularis mucosae of the intestine. The oral suckers of some of the flukes within the tissues were oriented toward the lumen, suggesting that these flukes might have been in the process of leaving the tissues since flukes were also found to have their posterior ends in the tunica propria and the oral sucker outside the tissue. When the worms were found within the tunica propria, the tissue was usually greatly compressed. The villi were compressed and appeared to be severed wherever the trematodes were in contact with them. The epithelium of the villi as well as the tunica propria was sometimes drawn into the oral sucker. When the flukes were found in the lumen of an intestinal gland, the glandular epithelium became flattened. Sometimes a portion of the glandular epithelium was completely eroded. Very often the worms partly within the glands were constricted by the opening of the glands.

The spiny gonotyl probably aided in damaging the tissue. These structures were extended in many worms so as to compress the tissue around them and erode the epithelium.

No flukes were found in serial sections of the intestines of chicks sacrificed 20 days after the initial feeding, although there were a few parasites in the lumina of the non-sectioned portions of the intestines of hosts sacrificed 20 to 25 days after infection. Microscopic studies of the intestines of these chicks showed that the tissues were of normal appearance. The glandular epithelium was cuboidal. No eggs or fragments of flukes were found in the tissue.

Of the 6 chicks that were fed 4 to 25 fish brains daily, 3 died before they were 2 weeks old. One of these which had been given chick feed only died when 8 days old. Another bird fed oatmeal only was sacrificed when 8 days old for comparison. A total of 537 worms were recovered from the last 10 mm. of the small intestine of the chick fed oatmeal and 214 from a similar area in the bird fed chick feed. Microscopic study revealed many worms between the villi as well as in the lumen. Two chicks fed almost daily with infected brains for 42 days were sacrificed on the 43rd day of the experiment. Only 4 parasites were found in a cecum of one chick and 28 flukes in a cecum of the other. No parasites were found in any other portion of the intestine. Sectioned material revealed no parasites or ova.

From these data it is evident that the young *E. californiensis* is capable of in-



FIGS. 1 to 4. Four non-adjacent successive sections of chick intestine showing invasion by a 15-hour-old fluke (arrow) in the tunica propria of a villus.

vading the tunica propria of the chick intestine. However, only a small number (1 to 2%) of flukes was found in the tissue. This may indicate that not all young flukes invade tissue, and that their stay in the tissue is very brief, thus suggesting that not all heterophyids require an intramucosal stage of development as concluded by Ciurea (1924). Those which did invade tissue showed no sign of staying in it but eventually escaped to the lumen. Thus *E. californiensis* differs from the heterophyid trematode studied by Africa and his associates who reported that once a fluke invaded the tissue, it did not return to the lumen but remained there until death and disintegration, after which time its eggs entered the general circulation.

It was observed in this study that once the chick overcame the infection, the parasites were eliminated despite continuous feeding of a large number of infected brains. The chicks underwent a "self-cure." This agrees with Willey and Stunkard's (1942) observation of *Cryptocotyle lingua* in terns and dogs, although they found no evidence of tissue invasion by that species.

SUMMARY

1. Serial sections of the intestines of experimentally infected chicks showed that 67% of *Euhaplorchis californiensis* were found between the villi.

2. Young flukes were capable of invading the intestinal wall of chicks. However the number of worms accomplishing invasion was small. These parasites apparently did not remain in the tissue, but eventually returned to the intestinal lumen.

3. It appeared that once the chicks overcame the infection, the flukes were eliminated despite continuous feeding of a large number of infected fish brains.

Literature Cited

Africa, C. M. 1937 Evidence of intramucosal invasion in the life cycle of *Haplorchis yokogawai* (Katsuta, 1932) Chen, 1936 (Heterophyidae). J. Philip. Islands Med. Ass. **17**: 737–743.

———1938 An attempt to elucidate the filtration of eggs of certain heterophyid trematodes into the general circulation. Philip. J. Animal Indust. **5**: 187–201.

———, Garcia, E. Y., and de Leon, W. 1935 Intestinal heterophyidiasis with cardiac involvement: A contribution to the etiology of heart failures. Philip. J. Pub. Health **2**: 1–35.

———, de Leon, W., and Garcia, E. Y. 1935 Heterophyidiasis II: Ova in sclerosed mitral valves with other chronic lesions in the myocardium. J. Philip. Islands Med. Ass. **15**: 583–592.

———, ——— and ———1936a Somatic heterophyidiasis in fish-eating birds, I: Ova associated with chronic lesions in the pancreas of a sea gull (*Larus ridibundus* Linn.). Philip. J. Pub. Health **3**: 29–35.

———, ——— and ———1936b Somatic heterophyidiasis in fish-eating birds, II: Presence of adults and eggs in the bile ducts of the cattle egret. Philip. J. Sci. **61**: 227–233.

———, ——— and ———1936c Heterophyidiasis: III: Ova associated with fatal hemorrhage in the right basal ganglia of the brain. J. Philip. Islands Med. Ass. **16**: 22–26.

———, ——— and ———1936d Heterophyidiasis: IV: Lesions found in the myocardium of eleven infested hearts including three cases with valvular involvement. Philip. J. Pub. Health **3**: 1–27.

———, ——— and ———1937 Heterophyidiasis: V: Ova in the spinal cord of man. Philip. J. Sci. **62**: 393–399.

———, ——— and ———1940 Visceral complications in intestinal heterophyidiasis of man. III Internat. Congr. Microbiol. (New York Rep. Proc.): 447–449.

Ciurea, J. 1924 Heterophyidés de la faune parasitaire de Roumanie. Parasitology **16**: 1–21.

Faust, E. C. and Nishigori, M. 1926 The life-cycles of two new species of Heterophyidae, parasitic in mammals and birds. J. Parasit. **13**: 91–128.

MARTIN, W. E. 1950 *Euhaplorchis californiensis* n. g., n. sp., Heterophyidae, Trematoda, with notes on its life-cycle. Tr. Am. Micr. Soc. **69**: 194–209.

STUNKARD, H. W. AND WILLEY, C. H. 1929 The development of *Cryptocotyle* (Heterophyidae) in its final host. Amer. J. Trop. Med. **9**: 117–128.

WILLEY, C. H. AND STUNKARD, H. W. 1942 Studies on pathology and resistance in terns and dogs infected with the heterophyid trematode *Cryptocotyle lingua*. Tr. Am. Micro. Soc. **61**: 236–253.

YOKOGAWA, S. 1913 Ueber einen neuen Parasiten *Metagonimus yokogawai*, der die Forellenart *Plecoglossus altivelis* (Temminck) zum Zwischenwirt hat. Bildung einer neuen Gattung. Centr. Bakt. Parasit. Abt. Orig. **72**: 158–179.

THE LIFE HISTORY OF *COOPERIA PUNCTATA*, A NEMATODE PARASITIC IN CATTLE[1]

T. BONNER STEWART

INTRODUCTION

Of the four species of the nematode genus *Cooperia* that occur in domestic ruminants in the United States, *C. curticei* is primarily a parasite of sheep, whereas *C. punctata, C. pectinata,* and *C. oncophora* are principally parasitic in cattle. *C. punctata* is common in bovines throughout the country and has a high pathogenic potential. Inasmuch as the details of the life cycle of this parasite had not been previously ascertained, the writer undertook such a study in the hope of tracing the complete development of a nematode which, according to the views of agricultural parasitologists, has the capacity of profoundly influencing the health of cattle when present in them in large numbers.

MATERIALS AND METHODS

All calves used in the experiments were grade Jerseys procured within 24 hours after birth from local farmers. They were raised free of worm parasites in portable pens (Davis, Bowman and Porter, 1952). The calves were fed milk twice daily and had access to alfalfa hay and grain, which became their regular diet after weaning. Water and salt were available to the animals at all times. Before the calves were drenched with infective larvae, they were moved to stalls in a concrete-floored barn, which had been previously disinfected to preclude any extraneous infections with helminth parasites. The stalls were thoroughly cleaned and washed once a day as long as the experiment lasted. A concrete vat filled with lye solution was placed at the entrance to the barn for the purpose of disinfecting the boots of any one entering or leaving that building.

Larvae for experimental infection of the calves were obtained from sphagnum moss cultures of feces (Cauthen, 1940) from a calf having a pure infection of *C. punctata.* Larvae were isolated from the cultures by means of a Baermann apparatus. Infections were accomplished by suspending the larvae in water and squirting the suspension into the mouths of the calves by means of a 5-ounce rubber bulb

Received for publication, October 21, 1953.

[1] From the Regional Animal Disease Research Laboratory, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture, Auburn, Alabama.

Condensed from a thesis submitted as partial fulfillment of the requirements for the degree of Master of Science in Zoology, Alabama Polytechnic Institute. The author acknowledges the assistance of Dr. Dale A. Porter, Director, Regional Laboratory, under whose direction this study was made.

attached to a dissecting blowpipe. The number of larvae given each calf varied from 9,000 to 46,000.

To determine the size range and typical appearance of eggs when passed in the feces, and as seen in routine fecal examination of cattle droppings, most of the eggs used for measurements were floated up with zinc sulfate solution (sp. gr. 1.18), the usual medium employed in making eggs counts with the Stoll (1936) technic in this laboratory. Enough debris was present in the preparation under the coverslip to prevent distortion of the eggs by pressure from above. For comparison, some eggs were recovered from living females and placed in distilled water for measurement and others were measured in smears of fresh fecal samples in distilled water.

Preparasitic stages were obtained from cultures prepared by cutting up gravid females in physiological saline and adding to it animal charcoal and a small lump of helminthologically sterile feces. The larvae were drawn off in a small Baermann apparatus at frequent intervals.

For obtaining the parasitic stages, calves were necropsied at various intervals (1 to 12 days) following experimental infection. Measurements of preparasitic and parasitic stages were made on fresh, heat-killed specimens, unstained, and floating freely in distilled water under a coverslip. All drawing were made with the aid of a microprojector.

RESULTS

Egg. The egg has a thin shell surrounding the light brown cellular mass, usually in the 16-cell stage when laid. The poles of the egg are nearly alike and the sides are straight and parallel (Plate I, Figure A). This parallelism is characteristic of the genus and partially differentiates the eggs of *Cooperia* from those of *Ostertagia.* On the basis of measurements of 100 eggs, the average width was determined to be 36 microns, the length 79 microns, and the form index 43. The different media used when measuring did not affect the size or shape of the eggs. The measurements agree closely with those of Shorb (1939).

First-stage larva. When cultured at a temperature of 20–26° C., the eggs hatched in about 20 hours. The newly hatched larvae were about 318 microns long, and almost doubled their length before molting (Plate I, Figures B and C). The buccal cavity of the first stage larva is a simple tube leading into the esophagus. The latter is typically rhabditiform, ending at its junction with the intestine in a prominent bulbular dilation. The esophageal valves, located in the bulbular portion, were distinct.

Second-stage larva. The second-stage larvae were first seen approximately 30 hours after hatching, when cultured at 28° C., but were first seen 15 hours later in the cultures kept at 20–26° C. (Table 1). The newly-molted larvae averaged 619 microns in length. Very little morphological difference was observed between first and second stage, except in size. The second stage had a darker appearance than the first, due to the accumulated 'nutrient' granules in the intestinal cells. The posterior part of the esophagus was not as bulbous nor were the esophageal valves as prominent. The first lethargus began approximately 12 hours before the first ecdysis. A period of quiescence also preceded the third stage, but its duration was not accurately determined.

TABLE 1.—*Development of the larvae of* Cooperia curticei *and* Cooperia punctata

Hours after hatching	At room temperature Larval stage *C. curticei* Andrews (1939)	At 20–26° C. Larval stage *C. punctata* Present writer	At 28° C. Larval stage *C. punctata* Present writer
12	First lethargus	First lethargus	First lethargus
30	Second stage	First lethargus	Second stage
45	Second stage	Second stage	Second stage
75	Second stage	Second stage	Third stage
78	Second lethargus	Second stage	
90	Third stage	Second lethargus	
96		Third stage	

Third-stage larva. The infective, third-stage larvae were seen 75 hours after hatching in cultures incubated at 28° C., and after 96 hours in those kept at 20–26° C. In this stage the average length was 798 microns. The appearance of the sheath and the gradual reorganization in shape of the esophagus and buccal capsule were the first noticeable signs of the approaching third stage. The presence of a recognizable excretory pore and the distinct cellular arrangement of the intestine appeared later. This stage has a globe-shaped buccal cavity. According to Dikmans and Andrews (1933), the buccal cavity of *C. oncophora* is pear-shaped and that of *C. curticei* flask-shaped. The esophagus was narrow and broadened gradually towards its junction with the intestine. The oval cuticularized bodies on either side of the anterior of the esophagus were not as large or as easily seen as those observed in *C. oncophora* by the present writer, but were similar to the bodies described by Dikmans and Andrews (1933) for *C. curticei.* The esophagus was only slightly constricted where it passed through the nerve ring, and was sometimes bent ventrally at this point. In 100 specimens the average length of the esophagus was 159 microns; the nerve ring was 129 microns from the anterior end. The genital primordium was 11 to 18 microns long, ventrad to the fifth ventral intestinal cell, and at a point approximately opposite the junction of the fifth and sixth dorsal intestinal cells. This structure appeared as a fairly clear body, rounded on the ventral aspect, and flattened on the dorsal side where it abutted against the intestine. The intestine consisted of sixteen well-defined cells, eight dorsal and eight ventral. The first ventral cell was longer than its dorsal counterpart, and the eighth ventral cell was the most posterior (Plate I, Figure D). The tail was 71 microns long on the average, and bluntly rounded; the tail sheath, sharply pointed, extended an average of 65 microns beyond the tail. Measurements of the infective larvae of *C. curticei* and *C. oncophora* were made by Dikmans and Andrews (1933) and are recorded along the with the present figures for *C. punctata* in Table 2.

PARASITIC STAGES

Third-stage larva. Larvae recovered one day after infection were still in the third stage. Not all had reached the intestine; a small sampling of the contents of

TABLE 2.—*Measurements*[a] *of the infective larvae of* Cooperia punctata *and the corresponding measurements of* Cooperia curticei *and* Cooperia oncophora

	C. punctata Present writer	*C. curticei* Dikmans and Andrews (1935)	*C. oncophora* Dikmans and Andrews (1935)
Total length including sheath	736–889 (798)	711–850 (752)	804–924 (862)[b]
Length of esophagus	140–173 (159)	130–162 (150)	152–180 (161)
Anterior end to genital primordium	379–465 (420)	345–448 (379)	395–500 (445)
Length of tail	50– 82 (71)	58– 70 (64)	60– 80 (64)
Anus to posterior tip of sheath	106–149 (135)	124–150 (137)	97–122 (109)

[a] All measurements in microns.
[b] Figures in parentheses represent averages.

the abomasum yielded larvae, some of which had already exsheathed. The exsheathed larvae averaged 772 microns in length. They were smaller than the infective sheathed forms, due in part to the loss of the sheath. At this stage the genital primordium was slightly more posteriad than in the free-living third stage, and was about 223 microns anterior to the anus. Once molted, the tail was characteristically bent dorsally (Plate I, Figure E.) Two days following infection, the larvae were still in the third stage, but none were found in the contents of the abomasum. The lips and papillae of the future fourth stage appeared as several highly refractive areas at the anterior and posterior parts of the buccal capsule, similar to that described in the comparable stage of *C. curticei* by Andrews (1939). At this stage the cellular structure of the intestine was indistinct, being evident only in the anterior portion. The intestinal lumen was wider and more clearly visible than before, and the dark granules formerly seen in the intestinal cells had decreased in number. The specimens recovered three days following ingestion were still mostly in the third stage. A few larvae however, having already molted, were in the fourth stage and those that had not molted had a loose cuticle. The average length of those still in the third stage was 1.2 millimeters (Table 3). In the more

TABLE 3.—*Measurements*[a] *of parasitic larvae of* Cooperia punctata

Days after infection	3	4	6	8	10	12
Total length	1.22	1.41 (1.62)[b]	2.58 (3.22)	4.15 (5.39)	6.23 (8.05)	6.65 (8.40)
Width of body at anterior end	0.02	0.02	0.02	0.04	0.04	0.05
Width of body at base of esophagus	0.03	0.03	0.04	0.06	0.06	0.07
Length of esophagus	0.21	0.23	0.28	0.35	0.37	0.38
Anus to tip of tail	0.08	0.08	0.08	0.14	0.17	0.18
Genital primordium from anus	0.15	0.25 (0.20)	...	...	...	...
Length of vagina and muscular ovijectors	...	...	0.23	0.33	0.39	0.39
Vulva to anus	...	...	0.55	0.95	1.47	1.69
Length of spicules	...	...	...	0.16	0.17	0.18

[a] Measurements are given as averages of ten specimens and recorded in millimeters.
[b] When two figures are given, the first one applies to the male and the one in parenthesis to the female.

advanced forms the genital primordium was about 48 microns long, and located about 146 microns anterior to the anal opening. The shape of the genital primordium had progressed by this time from the slightly compressed button-like body of the infective larva to a fusiform structure which, at its widest point, occupied one-third of the body width.

Fourth-stage larva. The sex was unmistakable four days after ingestion (Plate I, Figures F and G), when the worms were in the fourth stage. A swelling was present in the male at the region of the anus, and the posterior end of the female tapered to a sharp point. The males averaged 1.4 millimeters in length and the females 1.6 millimeters. The genital primordium was about 253 microns cephalad to the anus in the males, and 200 microns in the females. Dense areas were seen in the posterior swelling of the males. Morphological differentiation of the parts of the female genital primordium was not possible, although a double row of cells, as described by Andrews (1939) in the equivalent stage of *C. curticei* was seen. Invagination of the body wall proximal to the central part of the genital primordium had already begun and could be seen in some specimens examined. Six days following ingestion, the fourth-stage males had an average length of 2.6 millimeters and the females 3.2 millimeters (Plate I, Figures H and I). The males had grown

in width posteriorly to such an extent that the swelling, which had been prominent earlier, was not appreciably wider now than the width of the body just anterior to the swelling. The spicular mass could be distinguished, but the structure of the spicules could not be made out. The sperm duct had established its posterior connection with the cloaca in some specimens observed. The whole course of the genital system could be followed in a few of the males examined. The most anterior part of the testes reached to a point not more than 1.5 to 2 millimeters posterior to the esophagus. The seminal vesicle joining the sperm duct and testes could be traced along part of its course, but was not well differentiated from the other body structures. The bursal rays were in the process of formation, and the posterior portion of the cuticle protruded as a clear, spike-like tail. The parts of the female genital system were easily recognized. The chitinous lips of the vulva were an average distance of 548 microns from the anus. The sphincters of the ovijector could be made out in only some specimens, and the cellular structure of the uterus was easily discernible. The attachment of the uterus to the non-muscular ovijector was visible as a half-moon of uterine cells adjoining the highly refractive ovijector. The average length of the muscular ovijectors, including the sphincters, was 233 microns. In some specimens, the lumen of the non-muscular ovijectors was visible. The ovaries were located about one millimeter posterior to the esophageal-intestinal junction. The loose cuticle found in many males and females was indicative of the forthcoming, final molt.

Fifth-stage young adults. Most of the worms recovered eight days following infection were in the fifth stage, only a few had not yet completed the fourth ecdysis. A large increase in the size of the helminths over the ones found at the end of the sixth day seemed to indicate that the last molt occurred early during the seventh day. The males averaged 4.1 millimeters in length and the females 5.4 millimeters. All parts of the genital system of both sexes were readily seen. The spicules of the males averaged 165 microns long and had already assumed some of the characteristic folds and concavities peculiar to these structures in the mature worm, appearing darker and more distinct than before. The rays of the bursa and the genital cone were seen. The vulva of the female was an average distance of 952 microns anterior to the anus. The sphincters of the ovijectors were already well developed. The muscular part of the ovijectors and sphincters combined had grown to an average length of 333 microns and their width occupied about half of the body diameter of the worm at the region of the vulva. The lumen of the ovijectors was distinct. By the end of the tenth day the males were about 6.2 millimeters long and the females approximately 8 millimeters. The spicules of the males had darkened considerably and measured an average of 174 microns in length. Eggs were already present in the uteri of some females and the average length of the ovijectors including the sphincters was 386 microns.

Mature worms were obtained from a calf killed twelve days following infection. Since the adults have been well described by Ransom (1911), a recapitulation of the morphological details will not be given here.

SUMMARY

The life cycle of *Cooperia punctata* is direct, conforming closely to that of *C. curticei.*

The eggs are light brown, in the 16-cell stage when passed in the feces, their length more than twice their width, and their sides straight and parallel.

The first-stage larva hatches in 20 hours at 20–26° C.; at 28° C., this larva molts 30 hours after hatching, after a lethargic period of about 12 hours.

The third-stage larva emerges 96 hours after hatching, its average length, including the sheath, being 798 microns.

On ingestion by a susceptible calf the larva exsheaths and can be found as a third-stage parasitic larva 24 hours after ingestion.

The larva reaches the fourth stage at the end of the fourth day following ingestion; the male is swollen at the region of the anus and the posterior end of the female tapers to a sharp point.

The fourth ecdysis takes place during the seventh day following infection; at this stage the parts of the genital system are all present and readily identifiable. By the tenth day, eggs are forming in the uteri and the nematodes are nearly mature in all respects.

References

Andrews, J. S. 1939 Life history of the nematode, *Cooperia curticei*, and development of resistance in sheep. J. Agric. Res. **58:** 771–785.

Cauthen, G. E. 1940 A method of culturing large numbers of *Haemonchus contortus* larvae from eggs in cattle feces. Proc. Helminth. Soc. Wash. **7:** 82–83.

Davis, L. R., Bowman, G. W., and Porter, D. A. 1952 Portable pens compared with other enclosures for control of diseases of dairy calves. Vet. Med. **47:** 485–490.

Dikmans, G. and Andrews, J. S. 1933 A comparative morphological study of the infective larvae of the common nematodes parasitic in the alimentary tract of sheep. Trans. Amer. Micr. Soc. **52:** 1–25.

Ransom, B. H. 1911 The nematodes parasitic in the alimentary tract of cattle, sheep, and other ruminants. Bull. 127 Bureau Animal Industry, U.S. Dept. Agric., 132 pp.

Shorb, D. A. 1939 Differentiation of eggs of various genera of nematodes parasitic in domestic ruminants in the United States. Tech. Bull. 694, U.S. Dept. Agric., 10 pp.

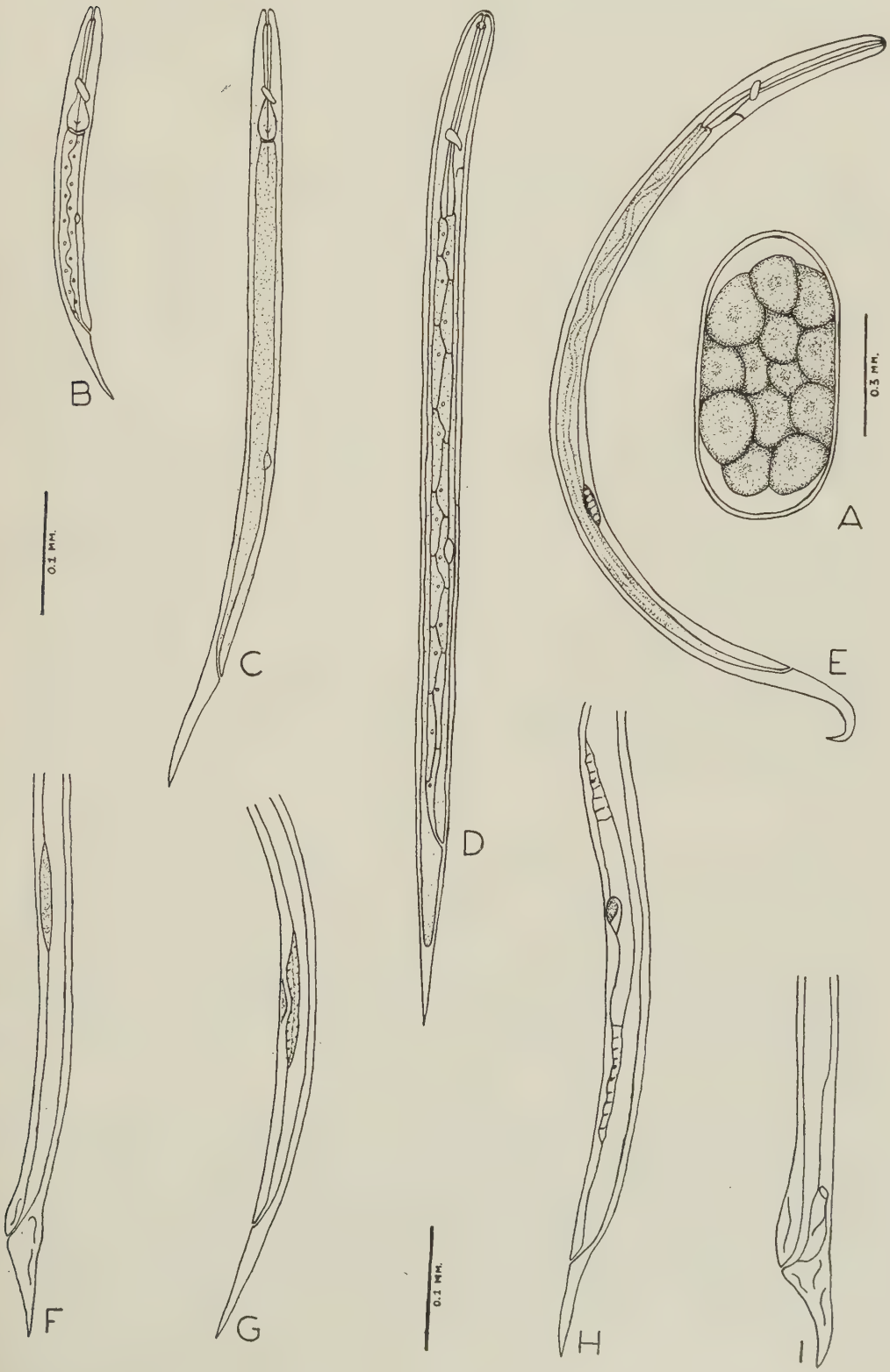
Stoll, N. R. 1936 Observations on cattle nematode infections, with a demonstration of their secondary transmission to grazing sheep. J. Parasit. **22:** 386–407.

Explanation of Plate I

(Drawn with the aid of a micro-projector)

Fig. A. Egg as passed in the feces.
Fig. B. First-stage larva, lateral view.
Fig. C. Second-stage larva, lateral view.
Fig. D. Third-stage larva, lateral view.
Fig. E. Parasitic third-stage larva in a typical position, lateral view.
Fig. F. Posterior end of early fourth-stage male, lateral view.
Fig. G. Posterior end of early fourth-stage female, lateral view.
Fig. H. Posterior end of advanced fourth-stage female, lateral view.
Fig. I. Posterior end of advanced fourth-stage male, lateral view.

PLATE I



ON THE LIFE CYCLE AND TAXONOMIC RELATIONS OF *CEPHALOPHALLUS OBSCURUS* N. G., N. SP., AN INTESTINAL TREMATODE (LECITHODENDRIIDAE) OF MINK

Ralph W. Macy and Donald J. Moore
Department of Biology, Reed College, Portland, Oregon

In 1943, the senior author discovered that all of the crayfish, *Astacus trowbridgii,* found in a creek in Multnomah County, Oregon, were heavily infected with an undescribed metacercaria. Examination of this crustacean host each year since has shown that the amount of infection has remained constantly high. Repeated attempts to infect guppies, guinea pigs, rats, day-old chicks, and ducklings all proved negative. Infected crayfish tails fed to a cat also produced no infection. In August, 1953, two raccoons at the Portland Zoo, previously found by fecal examination to be free of fluke infections, each were allowed to eat several heavily infected crayfish but repeated examinations of fecal samples were negative for trematode eggs. Shortly after this a mink was fed four, medium-sized, infected crayfish. Five days later, on August 26, fluke eggs were found in the feces; nine days later, when many dead flukes as well as eggs were being passed, the mink was killed and large numbers of adult flukes were recovered. Since the mink was ranch bred and previously had been kept on a diet of processed food free from crayfish, and since a careful check for possible previous infection had been made, it seemed safe to conclude that the trematodes came from crayfish fed during the course of the experiment. A second mink also was infected.

Meanwhile a virgulate cercaria from the snail, *Flumenicola virens,* from the same stream as the infected crayfish, was found to penetrate uninfected crayfish collected from elsewhere in the county, and the resultant metacercariae were identical with those capable of infecting mink. The fluke investigated in these experiments proved to be undescribed.

Acknowledgments

This work was supported by contract Nonr-153(00), NR 132-992, between the Office of Naval Research, Department of the Navy, and Reed College.

We wish to thank Messrs. Nirschl and Quinlin of the Nirschl and Quinlin Fur Farm, Portland, Oregon, for their kindness in supplying us with the mink used in these experiments.

Our gratitude also goes to Mr. Jack Marks of the Portland Zoo for making available several raccoons, and to Mr. William Tomori who helped make the collections and did other work.

Technique

Adult flukes were fixed in Gilson's fluid and stained with borax carmine or Ehrlich's acid hematoxylin. In addition to permanent whole mounts, serial sections and living specimens were studied for the more obscure details.

The stream snail *Flumenicola virens* does not live long without adequate aeration, but the problem of maintaining considerable numbers of isolated snails was solved by placing them individually in Syracuse watch glasses half filled with water.

Received for publication, October 12, 1953.

When stacked, the watch glasses were separated slightly by means of alternately placed toothpicks. Cercariae were studied alive both unstained and also stained with neutral red and Nile blue sulphate. Those used for some of the measurements were stained with neutral red and killed in hot 10% formalin. The life of the infected snails could be prolonged a week or longer by placing them in Petri dishes at room temperature or in Syracuse watch glasses in the refrigerator.

Metacercariae could be dissected from cysts with insect pins when the cysts were pushed against the closed points of small forceps. An easier technique was to place infected crayfish muscle in 0.01 to 0.05 molar NaOH which caused the cysts to double in size, become very thin walled, soften, and allowed the metacercariae to emerge. After this treatment they remained alive for as long as two days. Crayfish were readily maintained alive in aluminum trays built for the purpose and through which tap water flowed.

Cephalophallus obscurus n. g., n. sp. (Plates I & II)
(all measurements in millimeters)

Specific diagnosis: Body semi-cylindrical, 0.24–0.32 long by a width of 0.09–0.13 at the widest part, and covered with conspicuous T-shaped spines. Oral sucker subterminal, 0.05–0.053 in average diameter. Ventral sucker 0.05–0.06 in diameter, placed 0.11 to 0.14 from the anterior margin of the body. Pharynx averaging about 0.016 in diameter; prepharynx present. Esophagus of medium length and intestinal ceca short, reaching to the anterior margin of the testes. Testes round to oblong in outline, unlobed, averaging about 0.048 in diameter, overlapping the posterior part of the acetabular zone. Ovary rounded, 0.40–0.06 in diameter, lying on the right side, in the zone of the acetabulum or overlapping the anterior margin of the acetabulum. Seminal receptacle present, but variable in size, just posterior to the ovary. Laurer's canal apparently present. Vitellaria consisting of 12–16 follicles grouped laterally at the ends of the ceca and at the level of the acetabulum. Seminal vesicle beginning near the anterior margin of the ovary and extending across the midline of the body. Cirrus sac absent. Genital pore lateral and sinistral, at about the level of prepharynx, and surrounded by a slight elevation which forms a small genital papilla. Terminal half of protruded penis (one specimen) covered with minute spines (Pl. I, fig. 2). Ovary mainly postacetabular. Fully developed eggs 0.025–0.030 long by about 0.014 broad, brownish and operculate. Eggs in the feces of the mink frequently indented on one side and therefore possibly defective (Pl. II, fig. 11). Excretory vesicle an elongated sac.

Host of adult: *Mustela vison* Schreber.

Habitat: intestine, especially anterior portion of small intestine.

Locality: Multnomah County, Northwestern Oregon.

Type specimen: U.S. National Museum Helm. Coll. No. 49044, paratypes in authors' collection.

Cephalophallus, n. g.

Generic diagnosis: Lecithodendriidae: small flukes with heavily spined cuticula. Prepharynx, pharynx, and esophagus present. Intestinal ceca short, reaching only to level of acetabulum. Cirrus sac absent; seminal vesicle lying free in the parenchyma. Genital pore marginal, cephalad of the level of the pharynx. Cirrus with minute spines. Vitellaria near middle of body and lateral to acetabulum. Excretory vesicle saclike, not bifurcate.

Type species: Cephalophallus obscurus, n. sp.

Cercaria (Plate II, figs. 8, 9)

The cercaria of *Cephalophallus obscurus* is a virgulate xiphidiocercaria found in the snail *Flumenicola virens,* common in streams in western Oregon. The body of the flattened cercaria is about 0.18 long by 0.095 wide when alive, but averages 0.136 long by 0.079 wide when killed in hot formalin. The cuticula of the anterior half of the body is covered with minute spines. The oral sucker averages 0.048 in diameter but frequently changes shape when the animal is in motion; it is 0.045

long by 0.039 after treatment with hot formalin. In the living cercaria, the ventral sucker ranges from 0.023 to 0.025 in diameter, but averages 0.022 when preserved. When killed with hot formalin, the tail averages 0.14 long, but is usually much shorter in life except probably when swimming. In formalin-killed material, the acetabulum is about 0.067 from the anterior end of the body.

In this cercaria the virgula is of special interest and in newly emerged specimens this structure fills the posterior half of the oral sucker. It stains an intense orange color when exposed to dilute neutral red. Eighteen to twenty-four hours after the cercaria has been shed, the virgula is much diminished and may appear somewhat as seen in Fig. 9. The distinctive stylet is from 0.024 to 0.027 long and frequently changes position, being pushed forward, retracted, and moved in various directions in the living cercariae.

Four large and irregularly shaped penetration glands fill much of the posterior half of the body and are somewhat variable in position. Ducts from these lead forward and empty near the anterior margin of the oral sucker. At best, only a trace of any digestive tract can be found; a cluster of cells appears to form the fundament of the pharynx. Numerous unicellular glands scattered through the body hindered the study of the excretory system but its principle features were discovered. On each side of the body there are six pairs of flame cells so that the formula for this system is $2[(2+2+2)+(2+2+2)]$. From a knot of small excretory tubules at each side of the ventral sucker, a principal excretory duct leads to the anterior end of the sac-shaped excretory vesicle (Fig. 9).

It was found that the cercariae emerged almost entirely in the early evening, especially from about six to eight o'clock. They were immediately active, swimming near the bottom of the container or crawling on the bottom. Furthermore, they appeared to be slightly negative to light. The life span at room temperature was ascertained to be from about sixteen to twenty-four hours.

From 3% to 30% of the snails in the stream were infected, depending upon the location where they were collected. Two successive collections, totalling several hundred snails, yielded 17% and 28.3% infections.

Sporocyst (Plate II, fig. 12)

Development of the cercariae takes place within the oval sporocysts which measure up to 0.61 long by 0.35 wide. From one to four mature and active cercariae were observed in such sporocysts together with several in the process of formation.

Experimental Infection of Crayfish

A stream was found in which the crayfish, *Astacus trowbridgii,* were negative for trematode metacercariae. When placed in a container with an infected snail, cercariae were seen to be carried along the body of the animal by currents created by the swimmerets and other appendages. Although the cercariae showed no special attraction toward the host, when they came in contact with legs and other parts they did tend to attach and move in leach-like fashion over the surface. Actual penetration was not observed. Twenty-four hours after exposure, numerous tailless cercariae were found pushing slowly through the muscle tissue of the tail, especially that near the ventral side. Motion of the body was intermittent and progression was parallel with the striated muscle fibers. The stylet was retained for some

time after penetration (Pl. II, Fig. 6). Exposure of two other such crayfish to the cercariae was followed by the same course of infection.

Metacercaria (Plate I, figs. 4, 5; Plate II, fig. 7)

When removed from the cyst and studied in the living condition, the metacercaria appeared as in Fig. 4. The body is 0.3–0.34 long by 0.14–0.15 wide and is covered with prominent spines. The oral sucker measures approximately 0.06 in diameter, whereas the ventral sucker is slightly larger being about 0.065 in diameter and is placed near the center of the body length. The prominent pharynx is followed by an esophagus which divides into two short ceca. The primordia of the sex organs are readily seen. The sac-like excretory vesicle is somewhat variable in size. The flame cell pattern is similar to that of the cercaria, with six pairs of flame cells on each side of the body and with two main tubules leading from near the terminations of the ceca to the anterior end of the simple excretory vesicle.

Cysts containing the metacercariae are located primarily in the abdominal muscles of the crayfish but also occur in striated muscle in other parts of the body, but were not found in the muscle of the chelipeds and were absent from the gills. The oval cysts range from 0.12 to 0.40 long by 0.06 to 0.32 wide. Newly formed cysts are surrounded by a thin membrane of uniform thickness, whereas the larger cysts have much thickened ends (Fig. 7). When slightly compressed under a cover glass, the metacercaria within the cyst often shows intermittent movement.

From one medium-sized, naturally infected crayfish, 850 cysts were found in a 0.05 cc. sample. Since cysts tend to be rather evenly distributed in the abdomen, this would indicate the presence of about 17,000 cysts per cubic centimeter of muscle. In spite of the fact that all crayfish from the stream are infected and most of them carry an enormous burden of parasites, the population of these crustaceans is at least as great as it is in other comparable, local streams.

The high degree of resistance of the metacercariae is shown by the fact that they were still living two days after the death of a crayfish host which was kept at room temperature. Exposing the cysts *in situ* to 0.01 to 0.05 molar NaOH brought about the excystment of the metacercariae within a period ranging from a few minutes to several hours.

Natural infection of the crayfish begins soon after they hatch in early spring and there is a steady increase in cysts during the life of the host.

When fed to mink, most of the flukes matured within five days, but some were obviously immature. When the intestine was opened, at first no flukes were evident and only after remaining in normal saline solution for a period of fifteen or more minutes did they begin to emerge from between the villi. Sections of intestine show the flukes are all located in the base of the glands, near the *muscularis mucosae*. Typically the ventral sucker was attached to the epithelial cells (Pl. I, fig. 3).

The first mink used was heavily infected and began to show diarrhea and evident malaise just before being killed. Eggs of the flukes were concentrated in bits of watery mucous passed by the host. Although the infection may have caused the deterioration in health, change of diet also could have been the cause of the condition. The second mink infected was found at autopsy to have enlarged and tumorous kidneys but this condition probably was present before the time of infection with flukes.

Systematic Relations of *Cephalophallus obscurus*

The pattern of development, structure of the adult, and nature of the excretory system leave little doubt that *Cephalophallus* belongs to the Lecithodendriidae, and the position of sex organs and sac-like excretory vesicle indicate that it may be referred to the Allassogonoporinae, listed by Skrjabin (1948) to include the genus *Allassogonoporus* established by Olivier (1938). The latter investigator made no final decision as to its exact position in the family. Olivier pointed out the uncertainties regarding the limits and relationships of the two families Lecithodendriidae and Troglotrematidae, and stated further that the allocation of genera in these groups can be placed on a sound basis only when more data from life cycles has accumulated. Information gained in the present work strengthens the belief that *Allassogonoporus* should remain in the Lecithodendriidae in spite of the fact that it has a sac-like excretory vesicle characteristic of many of the Troglotrematidae, a condition also found in *Cephalophallus*. The flame cell pattern of the cercaria and metacercaria of *Cephalophallus* is the same as that of the corresponding larval stages of *Prosthodendrium chilostomum* (Mehlis), a bat fluke of the subfamily Lecithodendriidae, as recorded by Brown (1933). Further, a virgula is present both in the xiphidiocercaria of *Cephalophallus* and of *Prosthodendrium pyramidum* (Looss), the life cycle of the latter species having been described by Azim (1936).

The absence of a virgula in the cercaria of *P. chilostomum* suggests that this species should be removed from *Prosthodendrium* and placed in *Travassodendrium* Skarbilovich, 1943. Such a disposition is otherwise justified by the nature of the oral sucker and the vitellaria, although it was not so listed by Skrjabin (1948).

There are eight penetration glands both in the cercariae of *Cephalophallus obscurus* and of *Prosthodendrium chilostomum,* but only four in the cercaria of *Mosesia chordeilesia* (Pleurogenetinae) as determined by McMullen (1936), and no virgula is present in the latter species. A number of other virgulate cercariae are known, as mentioned by Kruidenier (1951), and when their life cycles have been investigated we may expect better to determine the relationships of the Lecithodendriidae.

It is of interest to note that in the several life cycles of the Lecithodendriidae which have been explored, only *Cephalophallus obscurus* has a crustacean second intermediate host; the other species utilize an aquatic insect for transport to the definitive host.

SUMMARY

A new genus and species of trematode, *Cephalophallus obscurus* (Lecithodendriidae: Allassogonoporinae) is described. Adults developed in mink, *Mustela vison,* which had been fed crayfish, *Astacus trowbridgii,* containing large numbers of metacercariae as a result of natural infection in a stream in Multnomah County, Northwestern Oregon. A virgulate xiphidiocercaria from an oval sporocyst in the snail *Flumenicola virens* was found experimentally to produce the metacercaria in the crayfish. Details of structure of the life cycle stages and taxonomic relations are discussed. *Prosthodendrium chilostomum* (Mehlis) is placed in the genus *Travassodendrium,* the new combination being *Travassodendrium chilostomum* (Mehlis).

References

Azim, M. A. 1936 On the life-history of *Lecithodendrium pyramidum* Looss, 1896, and its development from a xiphidiocercaria, *C. pyramidum* sp. nov., from *Melania tuberculata*. Ann. Trop. Med. Parasitol. **30**: 351–356.

Brown, F. J. 1933 On the excretory system and life history of *Lecithodendrium chilostomum* (Mehl.) and other bat trematodes, with a note on the life history of *Dicrocoelium dendriticum* (Rudolphi). Parasitol. **25** (3) : 317–328.

Kruidenier, Francis J. 1951 The formation and function of mucoids in virgulate cercariae, including a study of the virgula organ. Amer. Midl. Nat. **46** (3) : 660–683.

McMullen, Donald B. 1936 A note on the life cycle of *Mosesia chordeilesia* n. sp. (Lecithodendriidae). J. Parasit. **22** (3) : 295–298.

Olivier, Louis 1938 A new trematode, *Allassogonoporus marginalis*, from the muskrat. J. Parasit. **24** (2) : 155–160.

Skrjabin, K. I. 1948 *Trematoda* of animals and man, principles of trematodology. vol. 2. 600 pp., Moscow.

Explanation of Plate

Plate I

Cephalophallus obscurus, n. gen. and n. sp.

Fig. 1. Adult from intestine of *Mustela vison*, ventral aspect.
Fig. 2. Cirrus protruding from lateral body wall.
Fig. 3. Adult *in situ* as seen in section of small intestine of mink.
Fig. 4. Metacercaria, living and slightly flattened, dissected from cyst in the crayfish.
Fig. 5. Metacercaria, excretory system.

Explanation of Plate

Plate II

Cephalophallus obscurus (continued)

Fig. 6. Cercaria, after loss of tail, pushing through muscle of crayfish.
Fig. 7. Encysted metacercaria in muscle of crayfish.
Fig. 8. Cercaria, tail in partially contracted condition.
Fig. 9. Cercaria, excretory system. Virgula in nearly spent condition.
Fig. 10. Uterine egg.
Fig. 11. Egg from feces of mink showing the typical indented configuration.
Fig. 12. Sporocyst containing several completely formed and several developing cercariae.

PLATE I

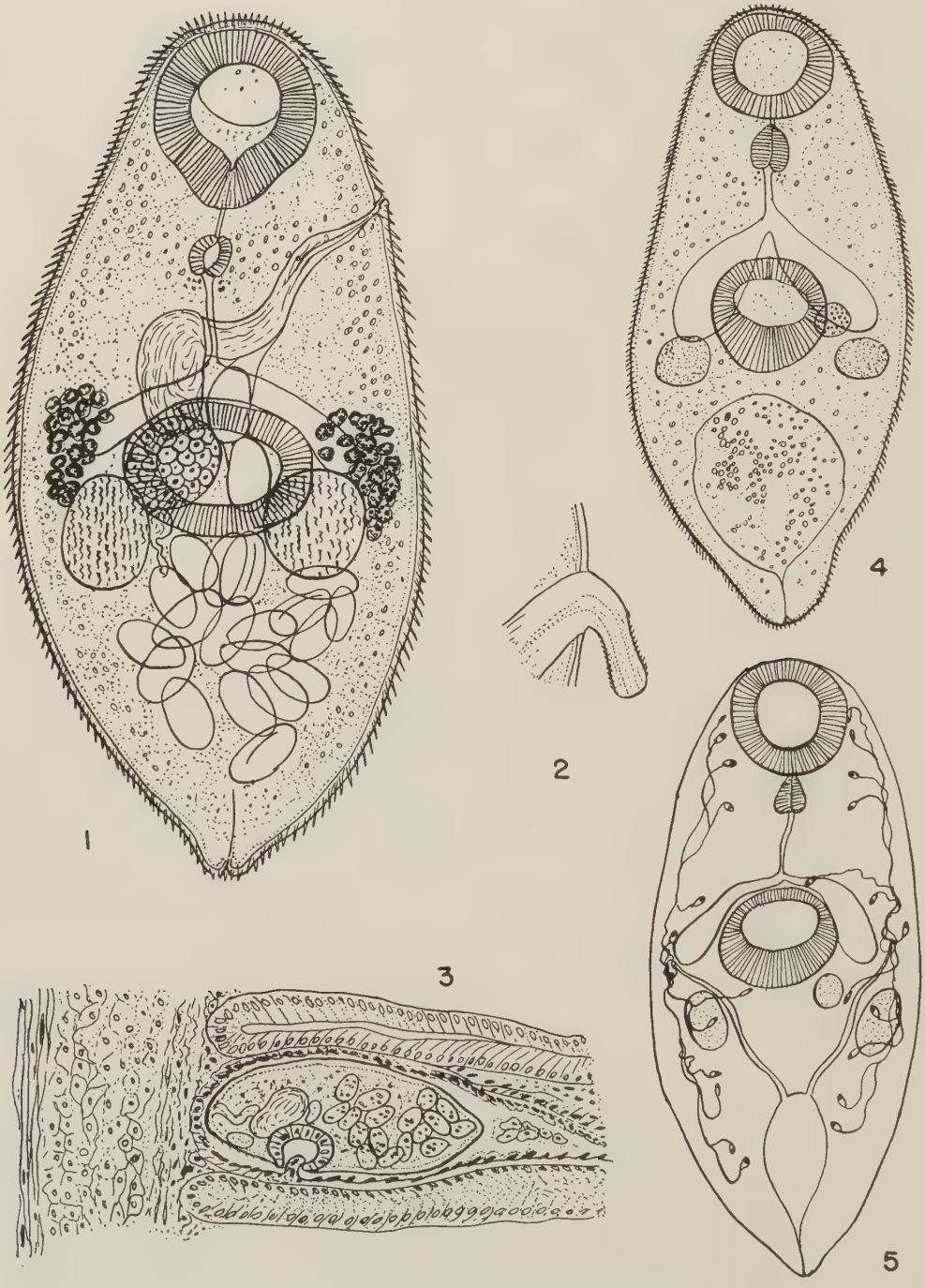
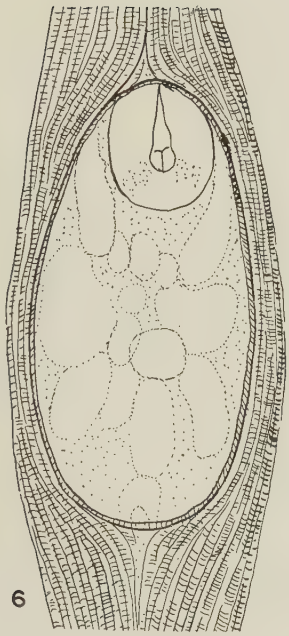
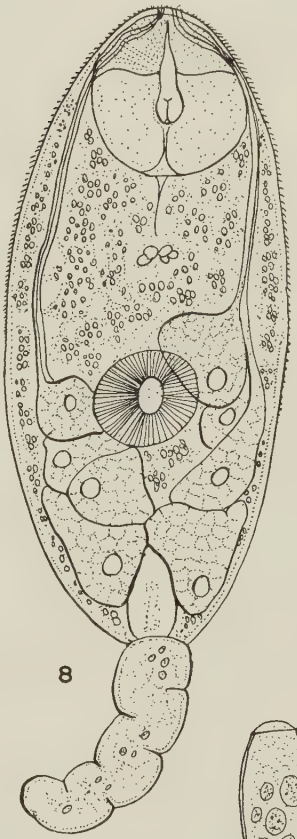
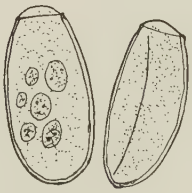
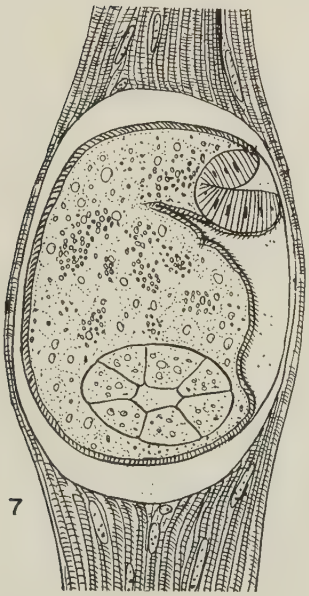


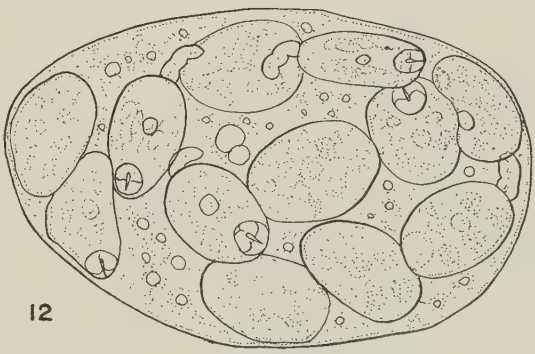
PLATE II











INFECTIONS PRODUCED BY MIXED STRAINS OF *PLASMODIUM GALLINACEUM* IN CHICKS

JOSEPH GREENBERG AND HELEN LOUISE TREMBLEY

U. S. Department of Health, Education, and Welfare
Public Health Service
National Institutes of Health, National Microbiological Institute
Laboratory of Tropical Diseases, Bethesda 14, Maryland

Little work has been done on the genetics of the *Plasmodia*. There are mechanical difficulties involved in isolating single clones, in differentiating genetically controlled characteristics, and in separating hybrids. We have for sometime maintained three strains of *Plasmodium gallinaceum;* all three were derived from a common source, but they have become differentiated over a period of about twelve years. This paper is concerned with two of these strains, the M and the BI.

The BI strain has been maintained exclusively by weekly blood passage for some nine years. Its peculiar characteristics have been described by Haas *et al.,* 1946, 1948; Lewert, 1948, 1950, 1950a; Greenberg *et al.,* 1950; Trembley *et al.,* 1951; and Richeson *et al.,* 1953. When transmitted by blood it produces a diphasic infection, *i.e.,* acute parasitemia, followed, in those chicks which survive, by an acute, usually fatal exoerythrocytic infection. During the stage of rising parasitemia, gametocytes, infectious to the mosquito (*Aedes aegypti*), are present in the circulating blood. Infections produced by the resulting sporozoites are exoerythrocytic exclusively; the chicks die of tissue infections before normal endoerythrocytic parasites appear in the circulation. The original diphasic pattern of the blood-passaged infection can be restored by serial passages of infected blood. The sporozoite-passaged infection is lethal for the parasite as well as the host since it cannot be transmitted by mosquitoes.

The M strain was originally isolated from the BI strain by Haas *et al.,* 1948 and subsequently by us (Trembley *et al.,* 1951a). This strain has been maintained in our laboratory for five years by cyclical mosquito-bird passage. When transmitted by blood the infection is monophasic; the acute parasitemia is low, about 80% of the chicks survive, and no exoerythrocytic infection follows. When transmitted by sporozoites the infection produces no detectable late tissue parasites.

We proposed to observe the results of serial mosquito-bird passage of mixed BI and M strain infections. Two assumptions were to be tested: (1) that the ability to produce late tissue forms and pigmented endoerythrocytic forms was genetically controlled and not linked; (2) that the two strains would cross. It was known that pure BI strain parasites could not be transmitted beyond the first mosquito passage. Should late exoerythrocytic forms be found in any of the chicks infected after the first mosquito passage this would be strong presumptive evidence of hybridization and genetic segregation of the characters.

METHODS

New Hampshire Red chicks from one supplier, weighing 45 to 60 grams served as hosts. Laboratory reared *Aedes aegypti* were used as vectors. The one mosquito equivalent dosage of sporozoites, described in detail by Coatney *et al.,* 1945

Received for publication, October 21, 1953.

and 1953, was used throughout. Methods of inoculating infected blood, preparing and reading blood and brain smears are described elsewhere (Coatney *et al.*, 1953). Blood smears were read daily beginning on day 7 (day of inoculation is day 0) on all chicks inoculated with sporozoites; examinations were discontinued when it was felt they no longer yielded pertinent information. Brain smears were made of all chicks at autopsy.

RESULTS

Seven chicks were each given intravenous inoculations containing 5,000,000 parasites of each of the two strains, M and BI. On the fifth day after inoculation *Aedes aegypti* females, in 3 lots of 50 to 100, were fed on each of three randomly selected chicks. Parasitemia in these chicks ranged from 70 to 80 per cent. Eleven days later sporozoites from each lot of mosquitoes were pooled and inoculated into each of 5 chicks per lot of mosquitoes. All of the sporozoite-inoculated chicks died of exoerythrocytic infections, but most of them had also developed some normal pigmented erythrocytic parasites (Table 1). One chick from each group was selected as being most likely to harbor mature gametocytes. One lot of 50 to 100

TABLE 1.—*Infections in chicks inoculated with sporozoites from mosquitoes fed on donors with mixed strain infections* (F_1)

Group No.	Chick No.	Parasitized RBC/10^4 RBC on day					
		7	8	9*	10	11	12
I	1	—	—		10	D++++	
	2	—	10		40	D++++	
	3	—	—		40	D++++	
	4	—	10		100**	D++++	
	5	5	10		D++++		
II	6	—	5		80	D++++	
	7	—	10		200	D++++	
	8	—	—		220	D++++	
	9	—	—		30	630**	D++++
	10	—	5		20	D+++	
III	11	5	10		770	D++++	
	12	—	5		D+++		
	13	—	5		D+++		
	14	—	5		10	D+++	
	15	—	5		260**	D++++	

— = No parasites detected.
D = Died. Pluses indicate prevalence of exoerythrocytic forms in brain smears made at autopsy.
* = Slides not read this day.
** = Mosquitoes fed at this time.

mosquitoes was fed on each of the 3 selected chicks. Sporozoites developed in the mosquitoes of each lot; nine to ten days later sporozoites from each lot were pooled and inoculated as above into each of five or ten chicks for each lot (Table 2). All of the 25 chicks so inoculated developed a normal parasitemia and none died of the infection; all were killed 29 days after inoculation. Brain smears of these chicks made at autopsy failed to exhibit exoerythrocytic parasites.

During the period of rising parasitemia two chicks from each group in Table 2 were selected and each furnished a blood meal to a single lot of mosquitoes. Thirteen to 16 days later the mosquitoes in each of the lots were found to harbor sporozoites. Three lots of mosquitoes, one from each of the groups, were selected arbitrarily to continue transmission of the parasite. The sporozoites from each of these lots were then pooled as usual and used to inoculate each of ten chicks per lot (Table 3). All of these chicks developed a relatively low grade parasitemia which

TABLE 2.—*Infections in chicks inoculated with sporozoites from mosquitoes fed on F_1 infections of Table 1 (F_2)*

Donor No.	Recipient No.	Parasitized RBC/10^4 RBC on day					
		7	8	9	10	11	12
	16	—	5	20	50	800*	880
	17	—	—	10	60	500	4,100
4	18	—	10	30	330*	4,000	4,500
	19	—	—	—	30	100	1,000
	20	—	—	20	240	630	2,350
	21	10	20	160	100	30	10
	22	—	10	70*	200	2,300	4,400
9	23	5	5	5	110	200	980
	24	5	156	590*	630	1,200	5
	25	—	5	10	150	800	4,500
	26	—	—	5	100	1,100*	3,500
	27	—	—	5	30	300	1,280*
15	28	—	—	—	10	40	120
	29	—	5	5	10	310	70
	30	—	—	5	10	200	1,150

* = Mosquitoes fed.
All chicks were killed day 29. No exoerythrocytic forms discovered in brain smears. The course of the infection is shown for only 15 chicks; there were 10 recipients from chicks 9 and 15. The infection did not differ materially in chicks not shown.

they survived. None were found to harbor exoerythrocytic forms when they were killed on the fourteenth day.

DISCUSSION

The results were essentially negative in terms of our original assumptions and aims. It is obvious that the first mosquito passage of the original "mixed" strain produced infections with characteristics of both. The exoerythrocytic forms of the BI strain were present in sufficient numbers to kill the chicks at the time expected if the chicks were inoculated with BI strain sporozoites only. Normal pigmented parasites and gametocytes, characteristic of the M strain, were also present. Mosquitoes fed on three chicks, admittedly selected because they were considered to harbor mature gametocytes, developed sporozoites. These sporozoites, and those from a subsequent serial mosquito passage, produced infections in chicks in no way distinguishable from pure M strain infections. There were no deaths from late exoerythrocytic forms. In fact, there was no evidence that such forms existed; the survival rate was 100 per cent. The ability to produce late exoerythrocytic parasites, characteristic of the BI strain, had disappeared.

Several explanations present themselves. (1) No hybridization occurred, the

TABLE 3.—*Infections in chicks inoculated with sporozoites from mosquitoes fed on F_2 infections in Table 2 (F_3)*

Donor No.	Recipient No.	Parasitized RBC/10^4 RBC on day			
		7	8	9	10
	31	—	—	10	50
	32	—	10	100	330
18	33	—	—	20	140
	34	—	—	10	60
	35	10	40	200	3,500
	36	—	10	100	1,000
	37	—	—	130	2,300
24	38	5	30	60	630
	39	5	10	100	2,000
	40	—	—	5	10
	41	10	60	500	4,000
	42	—	—	30	130
27	43	—	—	10	150
	44	—	10	50	1,300
	45	—	10	160	2,000

All chicks were killed day 14. No exoerythrocytic forms found in brain smears made at autopsy. The infection in only half of the chicks inoculated is shown. The remainder differed in no essential way from those in the table.

results of the first passage being the sum of the results of transferring each of the strains independently. If this is the correct interpretation, the question arises as to why hybridization did not take place especially in view of the fact that the M strain was derived from the BI strain in the first place. (2) Hybridization occurred but the characteristics of the BI strain were dominant so that all F_1 hybrids behaved as the BI parent. By the acceptance of this explanation one would have to assume that the ability to produce late exoerythrocytic parasites in abundance was linked with the inability to produce gametocytes, or that one gene controlled both characteristics. This last assumption makes it difficult to explain the behavior of the SP strain when it is transmitted through mosquitoes. This latter strain consistently produces abundant late tissue forms as well as pigmented trophozoites and gametocytes (James and Tate, 1938; James, 1939; Mudrow, 1940; Vitello, 1940; Huff and Coulston, 1944; Coulston *et al.*, 1945; Coatney *et al.*, 1945; Haas *et al.*, 1948; and Trembley *et al.*, 1951a). Furthermore, it is apparent that any malaria parasite which kills its host before it produces gametocytes is a poor parasite because it would be unable to survive in nature. Genetically the BI strain would be an aberrant mutant from the wild type and the chance of its being a dominant mutant is rare. (3) The characteristics being observed are not inherited through genes, but are possibly inherited through the cytoplasm. According to this explanation the BI strain is deficient in some protoplasmic substance which initiates the production of erythrocytic parasites, and which is furnished by M strain gametes when crossing occurs. This hypothesis leaves unexplained why the M strain produces no late tissue forms, and why the BI strain in blood passage produces erythrocytic forms.

It should be noted that if the M strain does not produce late tissue forms because of the immunity of the host, this immunity does not carry over to the BI strain when both strains are inoculated simultaneously.

SUMMARY

Chicks were infected by blood with two strains, M and BI, of *Plasmodium gallinaceum*. Sporozoites from mosquitoes, fed on these chicks, produced infections in which both late tissue forms and gametocytes were present. Chicks infected in two subsequent serial passages through mosquitoes showed no late tissue forms. The last infections were indistinguishable from those produced by the parent M strain, the characteristic of the BI strain of producing only late tissue forms having disappeared after the first mosquito passage.

References

Coatney, G. R., Cooper, W. C., and Trembley, H. L. 1945 Studies on *Plasmodium gallinaceum* Brumpt. III. The incidence and course of the infection in young chicks following the subcutaneous inoculation of pooled sporozoites. Am. J. Hyg. **42**: 323–329.

———, ———, Eddy, N. B., and Greenberg, J. 1953 Survey of antimalarial agents. Chemotherapy of *Plasmodium gallinaceum* infections; toxicity; correlation of structure and action. Pub. Health Monogr. No. 9, 322 pp. U. S. Government Printing Office, Washington, D. C.

Coulston, F., Cantrell, W., and Huff, C. G. 1945 The distribution and localization of sporozoites and pre-erythrocytic stages in infection with *Plasmodium gallinaceum*. J. Infect. Dis. **76**: 226–238.

Greenberg, J., Trembley, H. L., and Coatney, G. R. 1950 Strain differences in *Plasmodium gallinaceum* Brumpt. I. Differences in the behavior of the exoerythrocytic forms of

a blood-passaged (BI) and sporozoite-passaged (SP) strain of *Plasmodium gallinaceum*. J. Nat. Malaria Soc. **9**: 320–326.

HAAS, V. H., WILCOX, A., DAVIS, F. P., AND EWING, F. M. 1946 *Plasmodium gallinaceum* infections characterized by predominance of exoerythrocytic forms. Public Health Reports **61**: 921–928.

———, ———, LAIRD, R. L., EWING, F. M., AND COLEMAN, N. 1948 Symposium on exoerythrocytic forms of malarial parasites. VI. Response of exoerythrocytic forms to alterations in the life cycle of *Plasmodium gallinaceum*. J. Parasit. **34**: 306–320.

HUFF, C. G., AND COULSTON, F. 1944 The development of *Plasmodium gallinaceum* from sporozoite to erythrocytic trophozoite. J. Infect. Dis. **75**: 231–249.

JAMES, S. P. 1939 The incidence of exo-erythrocytic schizogony in *Plasmodium gallinaceum* in relation to the mode of infection. Tr. Roy. Soc. Trop. Med. & Hyg. **32**: 763–769.

——— AND TATE, P. 1938 Exo-erythrocytic schizogony in *Plasmodium gallinaceum* Brumpt, 1935. Parasitol. **30**: 128–139.

LEWERT, R. M. 1948 Alteration in the development of *Plasmodium gallinaceum* following passage through tissue culture. Science **107**: 250–252.

———, 1950 Alterations in the cycle of *Plasmodium gallinaceum* following passage through tissue culture. I. Tissue culture studies. Am. J. Hyg. **51**: 155–177.

——— 1950a Alterations in the cycle of *Plasmodium gallinaceum* following passage through tissue culture. II. The behavior of the strains during multiple passage through chicks. Am. J. Hyg. **51**: 178–193.

MUDROW, L. 1940 Klinische und parasitologische Befunde und chemotherapeutische Ergebnisse bei der Hühnermalaria. Arch. Schiffs-u. Tropen-Hyg. **44**: 257–275.

RICHESON, E. M., HIGHMAN, B., GREENBERG, J., AND COATNEY, G. R. 1953 Strain difference in *Plasmodium gallinaceum* Brumpt. V. Further characteristics of the BI strain. Am. J. Hyg. **57**: 114–124.

TREMBLEY, H. L., GREENBERG, J., AND COATNEY, G. R. 1951 Strain differences in *Plasmodium gallinaceum* Brumpt. II. Experiences with the sporozoite and single oocyst passage of the BI strain. J. Nat. Malaria Soc. **10**: 68–75.

———, ——— AND ———1951a Strain differences in *Plasmodium gallinaceum* Brumpt. III. The spontaneous conversion of a phanerozoite-producing SP strain to a phanerozoiteless M strain through mosquito passage. J. Nat. Malaria Soc. **10**: 76–81.

VITELLO, M. 1940 Azione del tartaro stibiato sul *Plasmodium gallinaceum*.. Ann. Med. Nav. Colon **46**: 481–485.

A NEW CYCLOPHYLLIDIAN CESTODE FROM THE AVOCET

DAVID T. CLARK

Department of Zoology, University of Illinois

INTRODUCTION

During a survey of the cestodes of birds from Nebraska two American avocets (*Recurvirostra americana* Gmelin) were taken, and from the small intestine of one avocet 40 specimens of a very unusual cestode were collected. The living worms were found free in the lumen of the intestine; organs of attachment were lacking in all specimens. External segmentation and the more obvious cestode structures were not apparent in the living specimens. Instead they presented a general appearance of slightly curved, extremely thin white ribbons with blunt ends of equal size. In physiological saline solution these delicate worms displayed weak, slow undulations. For this cestode a new genus and species, *Eurycestus avoceti*, is proposed.

MATERIALS AND METHODS

All specimens were fixed in A.F.A. Whole mounts were stained with Delafield's hematoxylin and a series of frontal and transverse serial sections 10 microns

Received for publication, September 9, 1953.

thick were stained with Mallory's triple stain. The description below is based in part on 15 whole mounts consisting of three immature specimens and twelve mature specimens, and in part on ten series of frontal serial sections and one series of transverse serial sections. All specimens were mounted in Permount.

DESCRIPTION

Eurycestus avoceti n. gen., n. sp.

The strobila consists of from 9 to 15 extremely short but very wide proglottids (Figs. 1 and 11), and a functional scolex is absent. A structure which appears to be a degenerate scolex was observed fortuitously in a frontal section through the most anterior portion of the strobila (Fig. 9). Thus it is believed that the scolex is degenerate in this cestode.

The relative age of succeeding proglottids is very clearly indicated by the width of the posterior proglottid margin which increases tremendously in each succeeding proglottid and is much wider than the anterior margin (Fig. 11). The anterior proglottid margin is only slightly wider than the posterior margin of the preceding segment, and the lateral margins form nearly a straight line with the anterior margin. The greatest width of the strobila is usually the width of the oldest, completely normal proglottid which may measure up to 29 mm. in mature strobilae. Frequently the oldest 1 or 2 proglottids are aberrant being narrower, incomplete medially and with poorly developed reproductive organs (Figs. 14 and 15). The length of the strobila is approximately 0.8 mm. at the midline where the length of all proglottids are included except for the one or two aberrant proglottids which may be present. The length of the mature proglottids is from 0.10 to 0.22 mm. at the region of each vitelline gland, but at the midline is less, being 0.05 to 0.18 mm. This reduction in length medially also occurs in younger proglottids which makes it possible for the total length of 10 proglottids at the midline to be approximately the same as the combined length of the 2 oldest proglottids through the region of the vitelline gland. The combined length of varying numbers of proglottids at different points is approximately 0.5 to 0.8 mm. The interproglottid margins are indistinct, especially in the region of the reproductive organs, and membranous partitions between the proglottids are lacking (Fig. 10). The musculature is very poorly developed consisting in mature proglottids of a few dorsal and ventral longitudinal fibers (Fig. 10). Calcareous corpuscles are abundant in older proglottids.

The osmoregulatory system is most easily observed in frontal sections; but this system can be observed in older proglottids of whole specimens. It consists of 2 extensive canalicular networks running in frontal planes in the medullary zone ventral to the reproductive system. The canals forming the network in the ventral plane are larger, of more variable caliber and more branched than the canals forming the network in the dorsal plane (Fig. 12). The ventral canals measure approximately 0.0035 mm. and the dorsal canals 0.0015 mm. in diameter.

Two complete sets of reproductive organs develop in each segment (Fig. 1). Rudiments of the cirrus pouch, cirrus and vagina first appear in proglottids 4 and 5 as darkly staining cell aggregates near each lateral margin, and the development of these structures is generally well demonstrated in successive stages posteriorly (Figs. 2–8). A short cirro-vaginal canal opens at the genital pore laterally on each

side just posterior to the anterior proglottid margin. In immature proglottids this canal is clearly common to the vagina and cirrus (Figs. 5 and 6).

In each of the two sets of reproductive organs of a proglottid there is a group of 30 to 50 spherical testes which measure on the average 0.060 mm. by 0.050 mm. The testes of each group are distributed in the posterior half of the proglottid where they extend from a point slightly lateral to the genital pore medially to the vitelline gland (Fig. 15). Frequently as many as 10 testes of a group may extend posteriorly into the anterior portion of the adjacent posterior proglottid. Vasa efferentia were not observed in any specimens, but a highly coiled vas deferens is present in each genital complex immediately median to the cirrus pouch (Fig. 15). The somewhat muscular cirrus pouch measures 0.148 to 0.315 mm. long and 0.035 to 0.060 mm. wide. The cirrus is long, well developed and strongly spined (Fig. 8). The everted cirrus measures 0.660 to 0.810 mm. long, and approximately 0.028 mm. wide at its base and 0.020 mm. at its tip. A seminal vesicle is absent.

The vagina passes medially from the cirro-vaginal canal, and posterior to the cirrus pouch the mature vagina describes several loops (Figs. 7 & 8). The lumen of the vagina is large, measuring as much as 0.036 mm. in diameter at the wider regions, and cilia are present in the median 2/3 of the looped inflated portion. Just median to the cirrus pouch the vagina becomes straight and the lumen abruptly constricted into a very narrow tube which is the point of union between the vagina and the lateral end of the seminal receptacle (Fig. 15). The seminal receptacle passing anterior to the testes may reach at times a length of 1.0 mm.; it is a club-shaped tube gradually expanding into a sac at the median end (Fig. 15). The fundaments of the reproductive system which increase considerably in development in each succeeding proglottid clearly indicate that the vagina and seminal receptacle arise separately and join secondarily (Fig. 3). The ovary (Fig. 15) is large, frequently extending 2.0 mm. medially from the cirrus pouch. It is a two-winged structure connected anteriorly and medially by the isthmus. The ovary appears to be composed of closely associated oöcytes, and the ovary fills in between the testes where these two organs overlap. The region posterior to the isthmus and between the two wings of the ovary is occupied by a group of cells which stain lightly and surround the various regions of the oviduct. This lighter staining region is closed off posteriorly by a compact darkly staining vitelline gland (Fig. 13) which measures 0.050 to 0.070 mm. long and 0.165 to 0.290 mm. wide. A funnel-shaped oöcapt (Fig. 13), continuous with the ovarian capsule, is located at the ventral and anterior portion of the lightly staining group of cells. From the oöcapt the oviduct as a distinct tube, the germiduct, with a heavily ciliated lumen passes posteriolaterally to the median end of the seminal receptacle. At the median end of the seminal receptacle the germiduct turns dorsally where it is joined by an extremely short seminal canal from the seminal receptacle, and the oviduct continues as the fertilization canal turning from the junction with the seminal canal to the opposite side of the lightly staining area. The fertilization canal (Fig. 13) is of smaller caliber than the germiduct and does not appear to be ciliated. The fertilization canal passes along the anterior border of the vitelline gland, and it is joined by the yolk duct just before expanding into the uterus. The nuclei of the cells which form the germiduct and the fertilization canal stain very darkly. The pregravid uterus of each set of reproductive organs is dorsally situated extending medially and later-

ally from the fertilization canal as a tube with long sac-like outpockets from the anterior and posterior sides (Fig. 14). The two uteri of a single proglottid remain independent of each other. Unfortunately oncospheres were not present in any of the uteri of the specimens studied. A typical Mehlis' gland and oötype were not observed.

Host: Recurvirostra americana, American avocet.

Habitat: Small intestine.

Locality: 5 miles N. Lincoln, Nebraska, April 28, 1951.

Type material: Holotype, U. S. Nat. Mus. Helm. Coll. No. 49075. Paratypes consist of 14 toto mounts and a series of serial sections. Paratypes have been deposited in the collections of Dr. H. W. Manter, University of Nebraska; Illinois State Natural History Survey; the writer; and others.

Eurycestus n. gen.

Generic diagnosis: Small cestodes with functional scolex unknown. Proglottids very broad, but short, transverse axis many times greater than longitudinal. Proglottids, acraspedote, few, delimited by weak musculature consisting of few fibers in each individual proglottid. Osmoregulatory system an extensive network throughout, in two frontal planes. Two complete sets of reproductive organs per segment. Cirro-vaginal canal opening in anterior portions of lateral borders. Testes numerous, situated in two posterolateral groups in each proglottid. Cirrus and cirrus pouch well developed, directed medially from each cirro-vaginal canal. Vas deferens much coiled, median to cirrus pouch. Seminal vesicle absent. Ovary large; two-winged, extending medially from each cirrus pouch. Vitelline gland compact, posterior to middle of ovary. Vagina of large caliber, coiled or looped, posterior to cirrus pouch. Seminal receptacle long, club-shaped. Junction of vagino-receptacular lumen very constricted. Genital ducts dorsal to osmoregulatory canals. Pregravid uterus a transverse tube with sac-like outpocketings, two per segment. Type species: *Eurycestus avoceti.*

DISCUSSION

The genus *Eurycestus* displays several morphological features which make its placement in the order Cyclophyllidea relatively certain even with the lack of knowledge concerning its scolex and oncospheres. These features are the marginal position of genital pores, the two-winged ovary, the single compact vitelline gland posterior to the ovary, and the absence of uterine pores. The placement of this genus into a family is not so easily accomplished, however.

Of the characters unique for this cestode, the most obvious is the great lateral extension of the posterior proglottid margin causing a strobila to be formed with its width many times its length. Another unique difference is the structure of the osmoregulatory system. The osmoregulatory system in *Archigetes* described by Wisniewski (1930) as a network, differs from that of *Eurycestus* by being composed of short canals running in all directions through the parenchyma rather than being composed of two networks in two distinctly separate frontal planes as in *Eurycestus.* The network of transverse canals in *Oochoristica* as described by Steelman (1939) and Peery (1939) is not as highly branched as that in *Eurycestus,* and it is clearly a communication between the ventral longitudinal canals. It appears that the network of osmoregulatory canals in *Eurycestus* is a modification to accommodate the great lateral extension of the proglottid which would leave the central portion of the proglottid too far from the usual laterally situated canals for osmoregulatory control were this network lacking. It is interesting that this system retains the larger ventral canals which is typical for cestodes.

The reproductive system is of little help in determining the taxonomic position of *Eurycestus*. Wardle and and McLeod (1952) list nineteen cyclophyllidian genera in several families which have partial or complete duplication of reproductive organs in the mature proglottids; however, only five of these (*Hepatotaenia* Nybelin, 1917; *Progamotaenia* Nybelin, 1917; *Pancerina* Fuhrmann, 1899; *Cotugnia* Diamare, 1893; and *Diplogynia* Baer, 1925) show both doubling of the uterus and two separate fields of testes. *Eurycestus* differs from all these in having a coiled vagina which has been reported from no other cestode at this time. Of these five genera only *Cotugnia* has its testes entirely posterior to the ovary as in *Eurycestus,* but *Cotugnia* differs from *Eurycestus* by having a much smaller and relatively poorly developed cirrus.

Thus it is seen that the genus *Eurycestus* has several characters which are found in several families, and the characters which are unique for this genus are not of sufficient rank to require a new family without more information concerning the scolex and oncospheres. At this time therefore, *Eurycestus* is not placed in a family.

SUMMARY

A description is given of *Eurycestus avoceti* n. gen., n. sp. from the small intestine of the American avocet (*Recurvirostra americana*). Its taxonomic position is briefly discussed. This new genus is in the cestode order Cyclophyllidea, but due to the lack of information concerning the scolex and oncospheres this genus is not placed in a family.

Addendum: In a recent paper (Lynsdale, J. A. 1953. On a remarkable new cestode, *Meggittina baeri* gen. et sp. nov. (Anoplocephalinae) from rodents in Southern Rhodesia. J. Helminth. **27** (¾): 129–142) a cestode is described which is superficially similar to *Eurycestus avoceti.* These two species are not congeneric, however, because of major differences in the reproductive systems.

LITERATURE CITED

PEERY, H. J. 1939 A new unarmed tapeworm from a spotted skunk. J. Parasit. **25:** 487–490.

STEELMAN, G. M. 1939 *Oochoristica whitentoni,* a new anoplocephalid cestode from a land tortoise. J. Parasit. **25:** 479–482.

WARDLE, R. A. AND MCLEOD, J. A. 1952 The zoology of tapeworms. Minneapolis. 780 pp.

WISNIEWSKI, L. W. 1930 Das genus *Archigetes* R. Leuck. Eine Studie zur Anatomie, Histogenese, Systematik und Biologie. Mem. Acad. Polon. Sc. et Lett., Cl. Sc. Math. et Nat., s. B: Sc. Nat. **2:** 1–160.

EXPLANATION OF PLATES

All figures are of *Eurycestus avoceti* n. gen., n. sp. Figures 1 and 11 were drawn with the aid of a projector, and all other figures with the aid of a camera lucida. All scales are in millimeters.

PLATE I

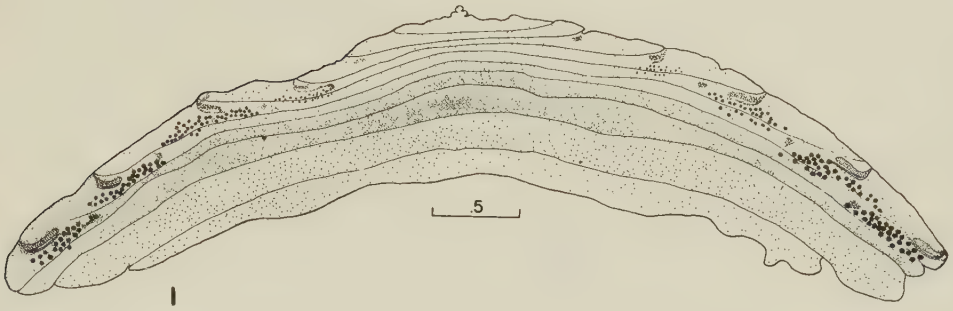
FIG. 1. Entire immature worm showing the arrangement of proglottids and the progressive development of the reproductive organs. Protandry is illustrated in this figure.

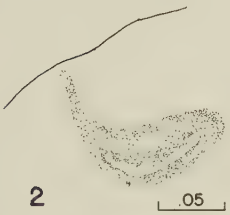
FIGS. 2–8. Various stages in the development of the cirrus pouch, cirrus and vagina; a series of stages usually present in a single strobila. The fundament of the seminal receptacle joins the forming vagina at approximately the stage shown in Fig. 3 where the entire fundament of the seminal receptacle is shown. Testes also appear at approximately this stage and are shown in this figure. Testes are omitted from figures of succeedingly older stages. Fig. 3 was drawn from a frontal section, all others from whole mounts. Figs. 3 to 8 are at the same scale as for Fig. 2.

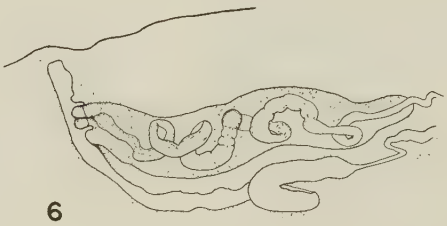
FIG. 9. Frontal section through the most anterior portion of a strobila showing a structure considered to be a degenerate scolex.

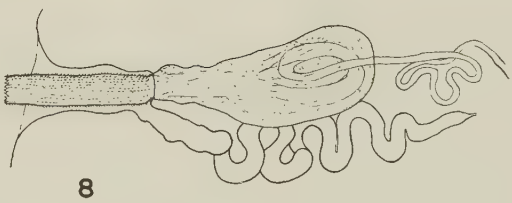
FIG. 10. Dorsal and ventral longitudinal muscles in three oldest proglottids and lack of membranous partitions between proglottids are shown in a longitudinal section through the median portion of a strobila where reproductive organs are lacking.

PLATE I









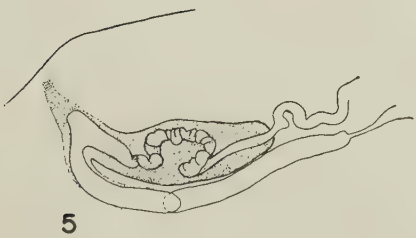


Plate II

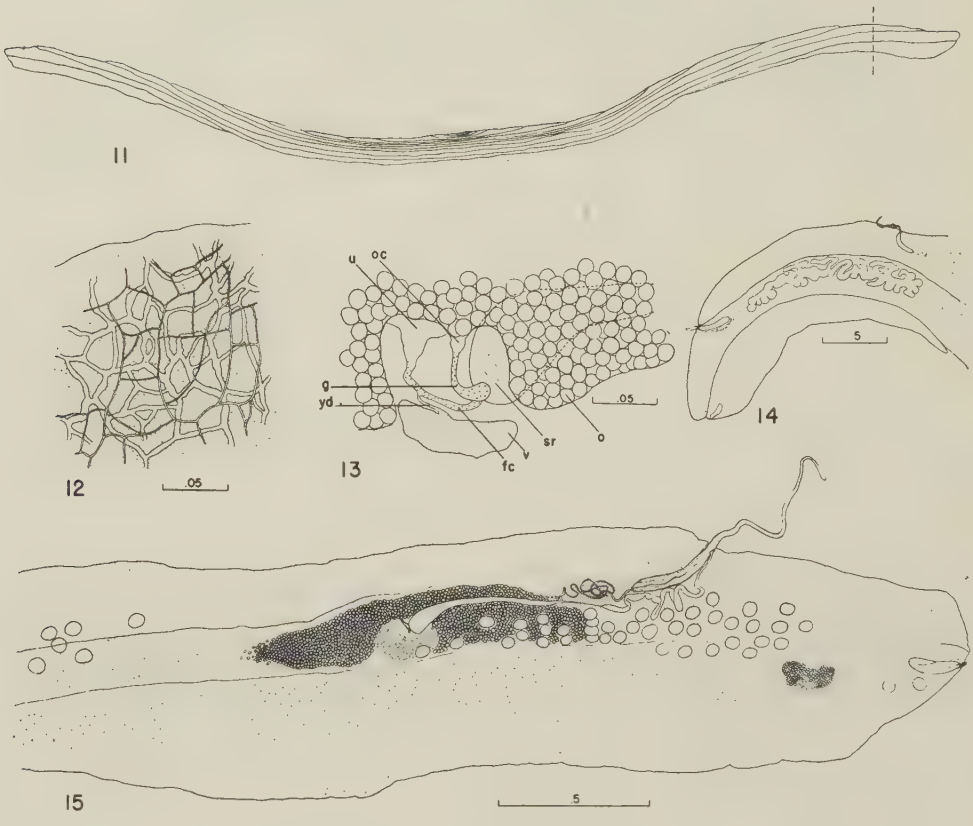


Plate II

Fig. 11. Mature strobila in outline showing the arrangement of the proglottids. The mounted specimen measures 29 mm. in width. The portion of the strobila to the right of the vertical line is illustrated in Fig. 15.

Fig. 12. Osmoregulatory network in a portion of a frontal section of a mature proglottid.

Fig. 13. Diagram of organs in interovarial space, ventral aspect. Lettering: fc, fertilization canal; g, germiduct; o, ovary; oc, oöcapt; sr, seminal receptacle; u, uterus; v, vitelline gland; yd, yolk duct.

Fig. 14. Pregravid uterus of one side of one proglottid shown in outline. Details of the other reproductive structures omitted. Aberrant posterior proglottid which is frequently incomplete medially is also shown.

Fig. 15. Reproductive organs of the portion of the strobila to the right of the line on the strobila illustrated in Fig. 11. Testes are represented by open circles; those to the left of the ovary belong to the adjacent anterior proglottid. The aberrant nature of the oldest proglottid is again indicated by the degenerate reproductive structures in the most posterior proglottid.

EFFECTS OF PENICILLIN ON *ENDAMOEBA GINGIVALIS* IN CULTURES WITH BACTERIA FROM THE HUMAN MOUTH*

JOHN P. CLAYTON, JR. AND GORDON H. BALL

Department of Zoology
University of California, Los Angeles

The great dependence of *Endamoeba histolytica in vitro* on other living cells is well known. For many years no successful culture could be maintained in the absence of bacteria, until the discovery that living *Trypanosoma cruzi* (Phillips, 1950; Phillips and Rees, 1950), could be substituted for the bacteria which continued survival of the endamoebae *in vitro*. Phillips and Rees found that growth of the amoebae was supported by trypanosomes, heated to 48° C. for 10 minutes but not by those subjected for the same length of time to 52° C. Recently Nakamura and Anderson (1951) showed that this ability to support growth at 48° C. was associated with respiratory activity of the flagellates at 48° C., even though they were apparently dead, while at higher temperatures both the ability to take up oxygen and to support the growth of the amoebae were lost. In 1952, Nakamura reported growth of *E. histolytica* up to 10 subcultures in an enzyme preparation free of other organisms. Shaffer, Sienkiewicz and Washington (1953) have found that *E. histolytica* will grow *in vitro* if fresh intact tissue cells from chick embryos are substituted for one-celled organisms.

On the contrary, *Endamoeba invadens* from reptiles has been grown with considerable ease apart from dividing cells (Miller, 1951; Lamy, 1948, 1951). Since this is also a tissue-invasive amoeba (Geiman and Ratcliffe, 1936), it seems improbable that dependence on dividing cells *in vitro* is directly connected with the parasitic habit of these endamoebae. In fact, in Miller's work, in which slices of fresh liver were added to sealed tubes of Ringer-Egg-Serum Medium, inoculated with *E. invadens,* it is doubtful if any liver cells would be viable for the time, a week or longer, that the amoebae grew in culture.

It is possible, of course, that the difficulty of maintaining *Endamoeba histolytica in vitro* apart from other living organisms may be a property common to many or all mammalian endamoebae. Among the latter, excluding *E. histolytica,* the majority are not commonly grown in culture and none have been reported as having been grown free of bacteria.

However, the commensal, *E. gingivalis,* can be cultivated with relative facility. Although it has considerable morphological similarity to *E. histolytica,* the differences in habitat of the two amoebae may possibly be correlated with differences in physiological requirements of the two species and be capable of expression when the amoebae are grown *in vitro.*

Apparently no attempt has been made to cultivate *Endamoeba gingivalis* free of bacteria. Howitt (1925, 1926) succeeded in cultivating this species by reducing the number of bacteria in the media with gentian violet, but this was rather a control of the bacteria in order to prevent their overgrowing the protozoa than an attempt to eliminate them. Koch (1926) and Kofoid and Johnstone (1929) using various

Received for publication, October 21, 1953.
* Aided by a grant from Board of Research, University of California.

dyes to control the associated bacteria found that bactericidal concentrations were lethal also to *E. gingivalis*. The employment of antibiotics, especially penicillin and streptomycin to bring about either bacterial elimination or bacteriostasis in protozoan cultures has provided a tool for testing the capacity of intestinal protozoa to grow independently of bacteria. Antibiotics are particularly valuable in freeing non-encysting protozoans from bacteria, for example *Trichomonas vaginalis* (Rees and Reardon, 1944; Johnson, Trussell and Jahn, 1945), where such techniques for removing bacteria as washing or the use of fairly strong bactericidal substances are precluded or are largely unsatisfactory. *E. gingivalis,* having no cysts, fits, consequently, into this category.

MATERIALS AND METHODS

Endamoeba gingivalis was obtained from the mouths of adult patients attending the Dental Clinics of the University of Southern California and the Wadsworth General Hospital of the Veterans Administration. Inocula were transferred directly to previously warmed culture media and transported in insulated containers to our laboratory.

Various media were employed. These included Howitt's modification (1925) of the Boeck-Drbohlav (1925) L.E.A. medium for the cultivation of *Endamoeba histolytica,* Johnson, Trussell and Jahn's (1945) denatured protein medium for growing *Trichomonas vaginalis,* and the mouth form *T. tenax* (Wainschel, 1952), gastric mucin which supports growth of *E. histolytica* (Ratcliffe, 1934) and *E. invadens* (Wirtschafter, 1952), and liver infusion agar commonly used to cultivate *E. histolytica* (Cleveland and Collier, 1930). Of these various media, Howitt's modified L.E.A. medium proved most successful and was employed as the standard stock medium. Since this mixture was also favorable for the associated bacteria at 37° C., transfers to fresh cultures were made daily. Cultures were carried in this medium for as long as three months and there was no indication that they could not have been maintained in it indefinitely.

The stock cultures were kept at a pH of 7.0–7.5, a range at which optimum growth was found to occur. When *Endamoeba gingivalis* and its associated bacteria were inoculated into tubes of Howitt's medium adjusted over a pH range of 5.5 to 7.5, growth of the amoebae took place, but the pH invariably returned to a point close to neutrality within 24 hours. As noted under the heading of results, there is strong evidence that this return to neutrality is due mainly to the effects of bacterial growth, since little pH change occurred with bacteriostatic concentrations of penicillin as long as the bacteriostasis persisted.

RESULTS

The minimum concentration necessary to achieve bacteriostasis was 1000 units of penicillin per cc. of medium. At this concentration, *Endamoeba gingivalis* ceased to multiply. A few amoebae might persist for 36 hours, but not beyond this period. The controls, on the contrary, inoculated at the same time and with the same volume of inoculum into the medium without penicillin showed rapid increase in numbers up to 32 amoebae per high power field in the first 24 hours and could still be detected in culture at 72 hours. Lower concentrations of penicillin were insufficient to produce bacteriostasis, and the growth of the amoebae was the same as in the control cultures. Amoebae failed to grow in the presence of 1000 units of penicillin

regardless of whether the cultures were inoculated directly from infected mouths or were subcultures taken at the peak of growth from strains flourishing on untreated media in the laboratory (Table 1).

It is possible that the associated bacteria may be necessary initially to "condition" the medium, and that such conditioned medium will support amoebic growth if the bacteria are then eliminated or prevented from multiplying. This does not seem to be the case, however, since if 1000 units of penicillin per cc. of medium are added to a culture of *Endamoeba gingivalis* at the peak of growth of the amoebae, bacteriostasis and the rapid decline of the amoeba culture ensue (Table 1).

Although Shaffer and Frye (1948) and others have found that concentrations of 1000 units of penicillin per cc. are not harmful to *Endamoeba histolytica in vitro,* it is of course possible that *E. gingivalis* in culture is more sensitive to penicillin than is *E. histolytica* and that the minimum concentration harmful to *E. gingivalis* may be practically the same as that needed to bring about bacteriostasis. In order to check on this possibility, cover slip preparations of *E. gingivalis* were prepared and maintained at 37° C. To one drop of the culture on the cover slip was added

TABLE 1.—*Effect of penicillin on growth of* E. gingivalis in vitro—*L.E.A. medium*

Age of culture in hours when penicillin added	Concentration of penicillin in units per cc. medium	Presence of *E. gingivalis* after penicillin is added			
		24 hours	36 hours	48 hours	72 hours
0	500	++++	+++	++	+
0	750	++++	+++	++	+
0	1000	+	+	0	0
0	1500	+	+	0	0
0	5000	0	0	–	–
0	7500	0	0	–	–
0 (control)	0	++++	+++	++	+
24	500	++	+	+	–
24	750	++	+	+	–
24	1000	+	+	0	–
24	1500	+	+	0	–
24	5000	0	0	–	–
24	7500	0	0	–	–
24 (control)	0	++	+	+	–

one drop of penicillin solution. In the controls, one drop of physiological saline was added to one drop of culture medium, physiological saline having been used throughout the experiments to dissolve the crystalline penicillin. In some instances, a small amount of neutral red was mixed with the penicillin solution and with the saline control in order to be sure that the material in this drop came directly and rapidly into contact with the amoebae.

At concentrations of 1000 units of penicillin per cc. as well as in the controls, no effect of either of the solutions was observed on the amoebae. Those in the drops containing penicillin continued to move about in a normal manner and persisted in the cover slip preparations, some of which were maintained anaerobically, for a period of eight hours. The controls, which had an abundant bacterial food supply, sometimes persisted longer. Since by eight hours, bacteriostasis was well established on the penicillin-treated slides, the absence of dividing bacteria is believed to be the cause of the disappearance of the amoebae. It is felt that any directly harmful effect on the protozoa would have been visible before this time. Nevertheless, as Bradin and Hansen (1950) point out for *E. histolytica* the con-

centration of a bacteriostatic agent necessary for direct amebacidal action cannot be determined until more is known of the relationship between bacteria and amoebae.

Even at concentrations of penicillin of 5000 units per cc., the amoebae showed no unfavorable effects directly from the antibiotic. When exposed to a concentration of 7500 units per cc. however, the amoebae were seen to cease movement in 10 or 15 minutes and they disintegrated very soon thereafter.

Since it was observed that pH 7.0 to pH 7.5 was optimum for growth of *E. gingivalis* in culture and since cultures inoculated into media with an adjusted pH range of 4.5 to 8.0 were found to have reached the optimum hydrogen-ion concentration after 24 hours, it is possible that the role of the bacteria is to produce and maintain a favorable pH.

To test this hypothesis, a set of culture tubes of pH 4.5 to 8.0 was inoculated from a culture of *Endamoeba gingivalis* growing well at a neutral pH. At the time of inoculation, penicillin was added to each experimental tube to produce a concentration of 1000 units per cc. At the end of 24 hours, the control tubes with no penicillin and an initial pH of 5.5 to 7.5 showed abundant growth and a pH of 7.0 to 7.5. The amoebae did not survive an initial pH of 4.5 or below or one as high as 8.0. The experimental tubes showed little or no change from the original pH and no

TABLE 2.—*Survival of* E. gingivalis in vitro *in L.E.A. medium with and without penicillin at different hydrogen ion concentrations*

Initial pH of medium	Concentration of penicillin in units per cc. medium	pH of medium after 24 hours	*E. gingivalis* after 24 hours	*E. gingivalis* after 48 hours
4.0	0	7.0	0	0
4.5	0	7.0	0	0
4.5	1000	4.8	0	0
5.5	0	7.0	++	+
5.5	1000	5.8	0	0
6.0	0	7.0	+++	++
6.0	1000	6.0	0	0
7.0	0	6.8	++++	+++
7.0	1000	7.0	+	0
7.5	0	7.2	++++	+++
7.5	1000	7.5	+	0
8.0	0	7.4	0	0
8.0	1000	7.8	0	0

amoebae were found in any of them except in those with an original pH of 7.0 to 7.5, where amoebae had persisted but had not multiplied (Table 2).

This indicates that although the associated bacteria were of value in bringing about a favorable pH in which the amoebae could increase in number, the optimum pH was not sufficient in itself to provide the necessary conditions for multiplication in the absence of bacterial reproduction.

It is worth noting that Wysocka and Wegner (1952) found *E. gingivalis in vivo* flourished with a diet of cocci and leptothrix, which occur normally in the human mouth.

DISCUSSION

The failure of *Endamoeba gingivalis* to grow and multiply in the absence of actively multiplying bacteria indicates that this amoeba, a commensal, is more comparable in its nutritional needs to the mammalian *E. histolytica,* a potential, if not an obligatory parasite *in vivo* rather than to *E. invadens,* a parasite of reptiles. With the exception of the work of Nakamura (1952), it has not been demonstrated

that *E. histolytica* can grow in culture in the absence of living cells of other organisms, such as bacteria or other protozoa, *e.g.,* trypanosomes, or fresh, intact cells from chick embryos (Shaffer, Sienkiewicz and Washington, 1953). *E. invadens* on the other hand can be propagated readily *in vitro* in the absence of dividing and probably even of viable cells, although apparently tissue fluids are essential for its continued culture. If the findings of Nakamura are confirmed that the *in vitro* growth of axenic *E. histolytica* is possible when succinic dehydrogenase and probably other enzymes are supplied, then the demand for these particular enzymes by other endamoebae should be investigated. Miller's work (1951) with *E. invadens* may indicate that such requirements are not necessary for all endamoebae. *In vivo,* large numbers of bacteria and spirochaetes favor the multiplication of *E. gingivalis* (Wysocka and Wegner, 1952), and it is perhaps less to be exepcted that it could grow free of actively multiplying cells than could *E. histolytica.* The *in vitro* culture of the parasitic *E. invadens* free of dividing, or probably of living, cells and the failure to cultivate the commensal *E. gingivalis* in the absence of actively multiplying bacteria lends some support to the contention of Hoare (1952) that *E. histolytica* has a commensal phase also, since *E. gingivalis* and *E. histolytica* both show dependence in culture on the presence of living cells.

SUMMARY

1. *Endamoeba gingivalis* did not grow *in vitro* if the associated bacteria were subjected to a bacteriostatic concentration of penicillin.

2. The concentration of penicillin used to produce bacteriostasis was not observedly harmful to *E. gingivalis.* This was evidenced by normal behavior and by a length of survival (8 hours) on cover slip preparations.

3. Although the actively dividing bacteria aid in producing an optimum pH *in vitro,* this is not their essential function for growth of *E. gingivalis,* since the latter will not multiply in cultures maintained at this pH in the absence of bacterial reproduction.

4. If the bacteria are essential for "conditioning" the medium, such conditioning must be of short duration since a medium supporting a flourishing culture of *E. gingivalis* becomes unsuitable for the growth of this amoeba after bacteriostasis is produced.

5. *E. gingivalis in vitro* is more like *E. histolytica* in its nutrition than it is like *E. invadens.* This may indicate the possible existence of an *in vivo* bacterial feeding phase of *E. histolytica.*

REFERENCES

BRADIN, JR., J. L. AND HANSEN, E. L. 1950 Indirect *in vitro* action of antibiotics in comparison with activity of accepted amebacides. Amer. J. Trop. Med. **30:** 27–41.

BOECK, W. C. AND DRBOHLAV, J. 1925 The cultivation of *Endamoeba histolytica.* Amer. J. Hyg. **5:** 371–407.

CLEVELAND, L. R. AND COLLIER, J. 1930 Various improvements in the cultivation of *Entamoeba histolytica.* Amer. J. Hyg. **12:** 606–613.

GEIMAN, Q. M. AND RATCLIFFE, H. L. 1936 Morphology and life-cycle of an amoeba producing amoebiasis in reptiles. Parasitology **28:** 208–228.

HOARE, C. A. 1952 The food habits of *Entamoeba histolytica* in its commensal phase. Parasitology **42:** 43–47.

HOWITT, B. F. 1925 The cultivation of *Endamoeba gingivalis* (Gros). Univ. Calif. Publ. Zool. **28:** 65–126.

————1926 The effect of certain drugs and dyes upon the growth of *Endamoeba gingivalis* (Gros) in vitro. Univ. Calif. Publ. Zool. **28:** 173–182.

JOHNSON, G., TRUSSELL, M., AND JAHN, F. 1945 Isolation of *Trichomonas vaginalis* with penicillin. Science **102**: 126–128.

KOCH, D. A. 1926 An experimental study of the effects of dyes, of dye mixtures, and of disinfectants upon *Endamoeba gingivalis* (Gros) *in vitro*. Univ. Calif. Publ. Zool. **29**: 241–266.

KOFOID, C. A. AND JOHNSTONE, H. G. 1929 The cultivation of *Endamoeba gingivalis* (Gros) from the human mouth. Amer. J. Publ. Health **19**: 549–552.

LAMY, L. 1948 Obtention d'une culture bactériologiquement pure d'amibes parasites pathogènes (*Entamoeba invadens*, Rodhain), ne comportant aucune addition de germes bactériens morts, ni d'aucun extrait microbien. C. R. Acad. Sci., Paris **226**: 2021–2022.

————1951 La culture pure des amibes parasites. J. Med. Bordeaux **128**: 253–256.

MILLER, M. J. 1951 A method for *in vitro* culture of *Entamoeba invadens* free from bacteria. Canad. J. Comp. Med. **15**: 268.

NAKAMURA, M. 1952 Substitution of an enzyme preparation for *Trypanosoma cruzi* in cultures of *Endamoeba histolytica*. Kitasato Arch. Exp. Med. **25**: 43–46.

———— AND ANDERSON, H. H. 1951 Effect of heat-treatment on the respiration of *Trypanosoma cruzi* used for the cultivation of *Endamoeba histolytica*. Amer. J. Trop. Med. **31**: 438–441.

PHILLIPS, B. P. 1950 Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*. Science **111**: 8–9.

———— AND REES, C. W. 1950 The growth of *Endamoeba histolytica* with live and heat-treated *Trypanosoma cruzi*. Amer. J. Trop. Med. **30**: 185–191.

RATCLIFFE, H. L. 1934 Gastric mucin as a culture medium for intestinal protozoa. Proc. Soc. Exp. Biol. and Med. **31**: 602.

REES, C. W. AND REARDON, L. V. 1944 Effects of penicillin on certain gram-positive bacteria growing in protozoan cultures. Trop. Med. News **1** (4): 18.

SHAFFER, J. G. AND FRYE, W. W. 1948 Studies on the growth requirements of *Endamoeba histolytica*. I. Maintenance of a strain of *E. histolytica* through one hundred transplants in the absence of an actively multiplying bacterial flora. Amer. J. Hyg. **47**: 214–221.

————, SIENKIEWICZ, H. S., AND WASHINGTON, J. E. 1953 The propagation of *Endamoeba histolytica* in tissue-bearing culture without accompanying bacteria or other microorganisms. Amer. J. Hyg. **57**: 366–379.

WAINSCHEL, J. 1952 Studies on *in vitro* cultivation of *Trichomonas tenax*. M.A. Thesis, UCLA.

WIRTSCHAFTER, S. K. 1953 Attempts to grow *Endamoeba invadens* free of bacteria. M.A. Thesis, UCLA.

WYSOCKA, F. AND WAGNER, Z. 1952 Occurrence of *Trichomonas tenax* and *Entamoeba gingivalis* in diseases of the oral cavity. Bull. State Inst. Marine and Trop. Med. Gdansk, Poland **4**: 290–291.

TISSUE RESPONSE TO *SCHISTOSOMA MANSONI* IN SECOND GENERATION SNAILS FROM A CROSS BETWEEN TWO STRAINS OF *AUSTRALORBIS GLABRATUS*

WALTER L. NEWTON[1]

U. S. Department of Health, Education, and Welfare
Public Health Service, National Institutes of Health
National Microbiological Institute,[2] Bethesda, Maryland

In previous studies with sectioned material (Newton, 1952), it was found that when miracidia of a Puerto Rican strain of *Schistosoma mansoni* penetrated young adults of a Brazilian strain of *Australorbis glabratus* the parasites were destroyed, usually within 24 to 48 hours after penetration. Marked cellular infiltration around

Received for publication, September 17, 1953.

[1] The author acknowledges with gratitude the assistance of Mr. Roy R. Reed, Laboratory of Pathology and Pharmacology, National Institute of Arthritis and Metabolic Diseases, for sectioning and staining the material used in this study.

[2] Laboratory of Tropical Diseases.

the parasites and a fibrotic type of walling-off process characterized this reaction. In contrast, when snails of a Puerto Rican strain of this species were exposed to the same parasite, normal development ensued, and there was no evidence of any reaction to the parasite. This behavior was consistent for each strain. With but a single exception, which did not appear to be developing normally, no parasite was found in the Brazilian snails without an accompanying cellular reaction, and all parasites found in sections of the Puerto Rican snails were free of cellular involvement.

In a study of the inheritance of susceptibility to this parasite in these two strains of snails (Newton, 1953), it was found that development of the parasite to the cercarial stage occurred in an average of 95 per cent of the Puerto Rican snails, 0 per cent of the Brazilian snails, and in intermediate proportions of subsequent generations from crosses between the two strains. Inasmuch as the marked difference in infection rate between the parent strains was associated with a similarly marked difference in tissue response to the parasite, it was of interest to observe the tissue response in snails of a subsequent generation derived from a cross between the two and which showed an infection rate somewhat intermediate between the two.

MATERIALS AND METHODS

Snails of F-2 generation XXIX-M, samples of which had previously shown an average infection rate of 33 per cent, were chosen for exposure to miracidia and subsequent sectioning. The parent of this population was a selfed nonsusceptible F-1 snail derived from a nonsusceptible Brazilian snail which had been fertilized by a susceptible Puerto Rican snail (see Newton, 1953). They had been reared in battery jars containing aerated tap water only and were fed on raw washed lettuce. The parasite strain was derived originally from Puerto Rico and had been maintained in the laboratory in white mice and in Puerto Rican *A. glabratus* for several years. It was the same strain that was used in the earlier study on tissue responses in the snail.

Twenty-nine young adult snails were exposed simultaneously to 20 miracidia each for several hours. These snails were treated with menthol and fixed in Bouin's 54 hours after exposure, according to a procedure described earlier (Newton, 1952). The head-foot region of each snail was removed, serially sectioned, and approximately two-thirds of this material was stained and examined under a compound microscope. A total of more than 3,000 sections was examined.

RESULTS

Of the 29 snails, parasites and relevant areas of reaction were found in 24. Among the latter, a total of 69 such items was found. This was a relatively low proportion of relevant findings in view of the fact that each snail was exposed to 20 miracidia. The average number of findings per snail was about 3, and no reaction areas or parasites were found in five of the snails. The possible failure of some miracidia to penetrate, and the fact that only the anterior portion of a snail was sectioned undoubtedly contributed to the low recovery. However, the latter does not compare unfavorably with the previous studies with the parent strains in which an average of 7, and in some specimens as few as 3, findings per snail were obtained in sections of the same area when 30 to 50 miracidia per snail were used.

The general histological findings were similar to those encountered in sections of the parent Brazilian and Puerto Rican strains after penetration by the parasite, and which were pictured in the earlier publication (Newton, 1952). The most prevalent single finding (32 of the 69) was a cellular aggregate with no recognizable remnants of the parasite. This had also been the most common finding in the parent Brazilian strain. In addition, there were 14 parasites which appeared to be developing normally, and around which no reaction was evident; this had been the characteristic finding in the parent Puerto Rican strain.

However, two other features were noted among the sections of these F-2 snails which were not observed in sections of the parent strains. First, there appeared to be considerably more variation in the condition of, and amount of reaction to, the other 23 parasites found than was true in the case of the parent Brazilian snails 2 days after exposure. So few cells were found around 11 of these parasites that it was difficult to determine whether a "reaction" was descriptive of the situation. This variation is of interest inasmuch as the reactions in the parent Brazilian snails were uniformly quite marked by the end of two days. The second feature was the distribution of the various findings among the 24 snails. Fourteen of the snails showed the "Brazilian" type of response, and five showed the "Puerto Rican" type, exclusively. However, in the other five snails both types of response were present. An apparently normal uninvolved parasite and one against which a cellular reaction had occurred were found in the same snail. As indicated earlier, both normal development and reaction were not found in the same snail in samples of either of the parent strains.

DISCUSSION

The facts that the response of some individuals of an F-2 population was similar to that characteristic of each of the parent populations, and that other individuals reacted in a manner which appeared to be somewhat intermediate indicate a variability in the F-2 population and suggest a possible relationship between snail genotype and tissue response. ("Tissue response" is used in its broadest sense to include possible humoral factors as well as the more obvious cellular aspects. It may be that the latter are secondary to other factors which initiate or bring about the death of the parasite.) Also, the fact that in some individuals a response occurred against one parasite and not against another suggests variability in the parasite population. Whether such is as fundamental as genetic variability or is expressive of merely a variability in the condition of the miracidia after penetration cannot be ascertained from the data at hand. In a study of the fate of miracidia of a Puerto Rican strain of *S. mansoni* in the tissues of *Tropicorbis havanensis* derived originally from Baton Rouge, Louisiana, Brooks (1953) also observed the apparently normal development of parasites in the same snail in which other parasites elicited a tissue reaction and were destroyed. He interpreted this finding as an indication of variation in the miracidia, and suggested that the exceptional normal development of a parasite in *T. havanensis* was due more to factors related to the individual miracidium than to the chance invasion of an especially susceptible snail.

One would anticipate that, in a given random population of either, variation in the susceptibility of the snails and in the potential of the parasites for survival and normal development would occur. The ultimate infection results following the mutual exposure of such populations would presumably depend, among other things, upon the relative strength of the susceptibility and developmental factors, their dis-

tribution among the individuals, and the eventual outcome of their interaction. Thus, the demonstration of the existence of variation in the one would seem to depend upon the background provided by the other against which it is measured and, of course, upon the fineness of the measurement. For example, as measured by a study of the parasite in sectioned snail tissues, variation among miracidia was not apparent when either the highly resistant Brazilian or the highly susceptible Puerto Rican snail population was used (Newton, 1952). In spite of the likelihood of some variation in susceptibility within each of these populations (Newton, 1953), individuals of the latter still provided backgrounds apparently constituted so strongly in the direction of susceptibility, or resistance, that variation in the parasite could not be detected. However, when a snail population, which was derived originally from a cross between the two extremes and which had an infection rate of 33 per cent, was used in the present study, the fate of the parasites in the tissues of certain individuals was suggestive of a variation in the miracidia. Such a snail population would presumably provide a more variable background of susceptibility than would either of the parent populations. Furthermore, some of the individuals would be expected to be of constitutions intermediate between the extremes of susceptibility and resistance shown by members of the parent populations. The susceptibility background provided by these individuals would appear, theoretically, to be the most suitable for detecting variation in the parasite. Thus, the *A. glabratus* in the present study, and the *T. havanensis* studied by Brooks, in which both normal development and destruction of the parasite occurred may have been snails of intermediate or "borderline" susceptibility.

SUMMARY AND CONCLUSIONS

Twenty-nine snails of an F_2 population derived originally from a cross between a susceptible Puerto Rican and a nonsusceptible Brazilian *Australorbis glabratus* were exposed to 20 miracidia of a Puerto Rican strain of *Schistosoma mansoni,* fixed 54 hours after exposure, and sections thereof were examined to determine the fate of the parasite.

Previous studies had shown that the parasite was destroyed in the parent Brazilian snails within 24 to 48 hours after penetration, and that there was a marked cellular reaction against the parasite. In the parent Puerto Rican snails, the latter developed normally, and no cellular response occurred. In the F_2 snails of the present study, tissue behavior characteristic of each of the parent populations occurred, along with reactions which gave the appearance of being intermediate between the two. In five of the snails, both normally developing parasites and those which elicited a cellular response, and were destroyed, were found in the same snail.

These findings suggested variation in both the susceptibility of the snail and the ability of the parasite to survive and develop. The problem of demonstrating the existence of variation in the parasite against a variable background of snail susceptibility is discussed.

REFERENCES

BROOKS, C. P. 1953 A comparative study of *Schistosoma mansoni* in *Tropicorbis havanensis* and *Australorbis glabratus.* J. Parasit. **39:** 159–165.

NEWTON, W. L. 1952 The comparative tissue reaction of two strains of *Australorbis glabratus* to infection with *Schistosoma mansoni.* J. Parasit. **38:** 362–366.

————1953 The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus.* Exp. Parasit. **2:** 242–257.

HELMINTH PARASITES OF EMBIOTOCID FISHES. I. A NEW GENUS OF THE TREMATODE FAMILY ZOOGONIDAE

Hisao P. Arai

Department of Zoology, University of California, Los Angeles

The parasitic fauna of the viviparous perches of the family Embiotocidae is not well known. The parasites reported in the available literature from hosts of this family are *Genitocotyle acirrata* Park, *Telolecithus pugetensis* Lloyd and Guberlet, and *Postmonorchis donacis* Young. The first named was described by Park (1937) from *Holconotus rhodoterus* Agassiz taken at Dillon Beach, California and also by Manter and Van Cleave (1951) from *Damalichthys vacca* Girard taken at La Jolla, California. Lloyd and Guberlet (1932) described *T. pugetensis* from *Cymatogaster aggregata* Gibbons taken in Puget Sound, Washington. *P. donacis* was described by Young (1953) from *"Micrometrus"* (undoubtedly *M. minimus* (Gibbons)), *"Cymatogaster"* (undoubtedly *C. aggregata* Gibbons), and from *Embiotoca jacksoni* Agassiz; all taken from Mission Bay, Calif. From one of the species of this family occurring in Japan, *Ditrema temmincki* Bleeker, Yamaguti (1934; 1939; 1942) has described *Decemtestis ditrematis* Yamaguti, *Stephanostomum ditrematis* (Yamaguti, 1939) Manter, 1947, and *Opegaster ditrematis* Yamaguti. From the same host, Ozaki (1929) described *Coitocaecum latum* Ozaki. Haderlie (1953) mentions the absence of helminth parasites in a single specimen of *Hysterocarpus traski* Gibbons (the only fresh-water embiotocid). This dearth of information is certainly not due to the rarity of the embiotocids. At least nineteen species of this family are well known components of the fish fauna of the California coast (Tarp, 1952).

A preliminary survey indicated that these fishes harbor a number of helminth parasites. A detailed parasitological study of these forms has been undertaken in hope of obtaining information which may aid in further understanding the taxonomy and ecology of the embiotocids by determination of apparent host specificity, as well as contributing to our knowledge of the helminth fauna of the Pacific.

During the examination of specimens of *Embiotoca jacksoni* Agassiz taken at Newport Bay and Morro Bay, California, 18 of 26 fishes examined harbored a trematode species belonging to the family Zoogonidae Odhner, 1911. A detailed study revealed that this form cannot be referred to any genus of that family, and it is concluded that the erection of a new genus is required.

Appreciation is expressed to Dr. Clark P. Read and to Dr. Boyd W. Walker with whose guidance and assistance this work was carried out.

Neozoogonus californicus n. g., n. sp.

Specific diagnosis: Body small, pyriform, widest at point just posterior to testes; 0.483 to 0.875 mm. long and 0.174 to 0.371 mm. wide, averaging 0.682 by 0.266 mm. Cuticle spinous, uniformly spined at anterior end but spines apparently lacking at posterior end; color white to deep orange. Oral sucker terminal, cup-shaped, with oral opening anteriorly directed, 0.042 to 0.086 mm. long and 0.112 to 0.165 mm. wide, set off from body by neck-like constriction. Ventral sucker usually subspherical, occasionally spherical, 0.053 to 0.106 mm. in transverse diameter, protruding in some individuals (due to pressure in mounting?). Prepharynx 0.056 to 0.080 mm. long; pharynx subspherical, 0.040 to 0.070 mm. in transverse diameter; esophagus slightly

Received for publication, October 3, 1953.

longer than prepharynx. Intestinal bifurcation just posterior to ventral sucker; ceca broad and long, extending posteriorly nearly to end of body.

Common genital pore on right ventral margin of body, near level of posterior edge of ventral sucker. Cirrus sac large, approximately 0.23 to 0.56 mm. long and 0.043 to 0.083 mm. wide, crossing mid-line diagonally and recurving to extend posteriorly to mid-line just antero-ventral to testes. Cirrus club-shaped, unarmed. Pars prostatica and seminal vesicle present in cirrus sac. Metraterm weakly muscular. Seminal receptacle ovoid, in mid-line, just dorsal and posterior to ovary, 0.029 to 0.062 mm. in transverse diameter; two accessory seminal receptacles present, one bladder-like, extending medially from uterus near metraterm, the other a more proximal bulbous swelling of uterus. Testes symmetrical and usually rounded, in middle of body, 0.061 to 0.104 mm. in diameter (averaging 0.090 mm.). Ovary subspherical, postero-ventral to testes, in mid-line, 0.066 to 0.138 mm. in transverse diameter (averaging 0.101 mm.). Vitellaria compact, just postero-ventral to ovary, 0.024 to 0.069 mm. in transverse diameter; uterus thin-walled, much convoluted, with ascending and descending limbs, tending to fill posterior portion of body. Mehlis' gland dorsal to vitellaria, ventral to seminal receptacle. Measurement of eggs and study of excretory system not feasible.

Host: Embiotoca jacksoni Agassiz, black bay perch.

Location: Intestine.

Localities: Morro Bay, California (type locality) and Newport Bay, California.

Incidence: In all of fourteen specimens of black bay perch examined from Morro Bay and in four of twelve specimens examined from Newport Bay, California.

Type specimen: U.S. National Museum, Helminthological Collection No. 49035.

Paratypes: Helminthological Collection, Department of Zoology, University of California, Los Angeles.

Neozoogonus n. g.

Diagnosis: Pyriform Zoogoninae with intestinal bifurcation posterior to ventral sucker. Ceca long and broad, almost reaching posterior end of body and with lateral margins extending to edges of body. Genitalia mostly intracecal and compact in spatial relationship. Cirrus sac very large, diagonally traversing nearly width of body. Oral sucker terminal with oral opening anteriorly directed. Accessory seminal receptacles present near metraterm.

Type species: Neozoogonus californicus n. g., n. sp.

DISCUSSION

The morphology of *Neozoogonus* indicates that it should be referred to the subfamily Zoogoninae Odhner, 1911. It resembles the species of the genus *Zoogonus* in having the intestinal bifurcation posterior to the ventral sucker. *Neozoogonus* differs from the species of *Zoogonus* in having broader and much longer ceca and much more compact genitalia. In addition the oral sucker differs in shape and in attachment to the body; in *Neozoogonus* the sucker is set off from the body by a neck-like constriction. The bladder-like structure extending from the uterus near the metraterm is similar to the structure described by Yamaguti (1938) in *Zoogonoides acanthogobii.* This saccular structure is assumed to be another accessory seminal receptacle since a bulbous swelling of the uterus of the type generally regarded as the accessory receptacle is also noted.

Three specimens, resembling *Neozoogonus californicus* in most respects except size, have been recovered from one specimen of *Taeniotoca lateralis* Agassiz, the striped sea perch. A complete description of the form from *Taeniotoca* is deferred until additional material is available.

REFERENCES

HADERLIE, E. C. 1953 Parasites of the fresh-water fishes of Northern California. Univ. Calif. Publ. Zool. **57:** 303–404.

LLOYD, L. C. AND GUBERLET, J. E. 1932 A new genus and species of Monorchidae. J. Parasit. **18:** 232–239.

MANTER, H. W. AND VAN CLEAVE, H. J. 1951 Some digenetic trematodes, including eight new species, from marine fishes of La Jolla, Calif. Proc. U.S. Nat'l. Mus. **101:** 315–340.

OZAKI, Y. 1929 Note on Coitocaecidae, a new trematode family. Annot. Zool. Japon. **12:** 75–90.

PARK, J. T. 1937 A new trematode, *Genitocotyle acirrus* gen. nov., sp. nov. (Allocreadiidae) from *Holconotus rhodoterus*. Trans. Amer. Micr. Soc. **56:** 67–71.

TARP, F. H. 1952 A revision of the family Embiotocidae. (The surf-perches.) Calif. Dept. Fish and Game, Fish Bull. No. 88.

YAMAGUTI, S. 1934 Studies on the helminth fauna of Japan. Pt. 2. Trematodes of fishes, I. Jap. J. Zool. **5:** 249–541.

————1938 Studies on the helminth fauna of Japan. Pt. 21. Trematodes of fishes, IV. Published by the author. Kyôto, Japan. 139 pp.

————1939 Studies on the helminth fauna of Japan. Pt. 26. Trematodes of fishes, VI. Jap. J. Zool. **8:** 211–230.

————1942 Studies on the helminth fauna of Japan. Pt. 39. Trematodes of fishes mainly from Naha. Trans. Biogeograph. Soc. Japan **3:** 329–398.

YOUNG, R. T. 1953 *Postmonorchis donacis*, a new species of monorchid trematode from the Pacific Coast, and its life history. J. Wash. Acad. Sci. **43:** 88–93.

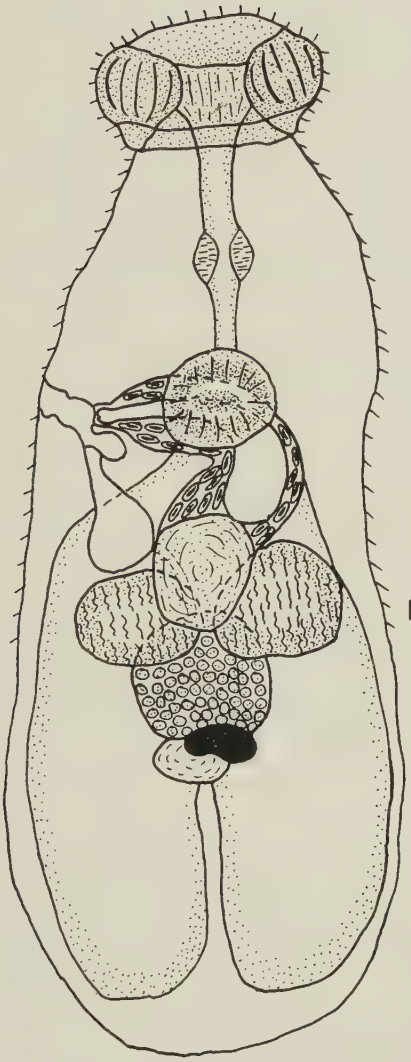
EXPLANATION OF PLATE

Both drawings were made with the aid of the camera lucida. The projected scales have the values indicated.

FIG. 1. *Neozoogonus californicus* n. g., n. sp., ventral view of whole mount with uterus omitted.

FIG. 2. Ventral view of genital complex of *Neozoogonus*.

PLATE I



0.1 mm.

FIG. 1

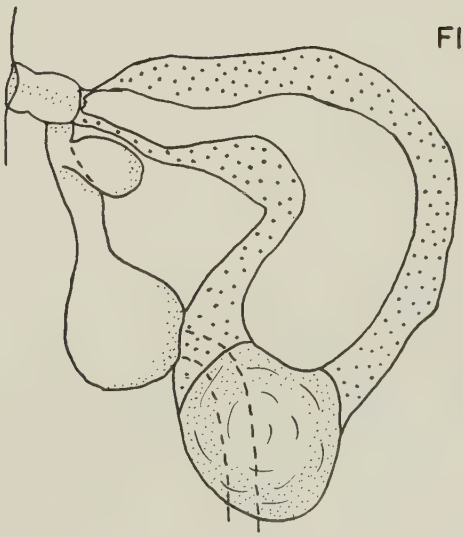


FIG. 2

0.1 mm.

EVIDENCE FOR THE EXISTENCE OF THE ENZYMES HEXOKINASE AND ALDOLASE IN THE PROTOZOAN PARASITE *TRICHOMONAS VAGINALIS*

Samuel K. Wirtschafter

Department of Zoology, University of California at Los Angeles

Although *Trichomonas vaginalis* has been available in culture free from associated microorganisms for a number of years, little work dealing with its metabolism has been reported. Trussel and Johnson (1941), reported the fermentation of carbohydrates and related compounds by *Trichomonas vaginalis*. Ninomiya and Suzuoki (1952), further extended this work by reporting the fermentative activity of *Trichomonas vaginalis* on compounds not previously investigated, as well as the optimum conditions for activity. However, to date, no account of the intermediary metabolism has been given.

The studies reported in this paper deal with the demonstration of two enzymes which are involved in phosphorylative glycolysis. These enzymes are: hexokinase, which in vertebrate tissue and yeast catalyzes the transfer of phosphorus from adenosine triphosphate (ATP) to glucose to yield glucose-6-phosphate and adenosine diphosphate (ADP); and aldolase, which catalyzes the breakdown of fructose-1,6-diphosphate to yield 3-phosphoglyceraldehyde and dihydroxyacetone phosphate.

The presence of these two enzymes in homogenates of *Trichomonas vaginalis* gives strong evidence for the existence of a phosphorylative carbohydrate degradation which is similar to that which is present in vertebrate tissue and yeast.

MATERIALS AND METHODS

Two strains of *Trichomonas vaginalis* were used in this study. They were isolated from clinical cases of trichomonad vaginitis, and designated as strains 2A and 5A. They were freed from associated microorganisms with the aid of penicillin and streptomycin. Stock cultures were maintained in the CPLM medium of Johnson and Trussell (1943), at 37° C., and subtransferred every 48 hours. Organisms desired for homogenate studies were grown in a modified CPLM medium in which the agar had been omitted and 0.1% sodium thioglycolate had been added. Cultures were grown in flasks for 48 hours at 37° C. in 100 ml. of medium. To insure complete anaerobiosis flasks were incubated inside an evacuated desiccation chamber.

Homogenates of *Trichomonas vaginalis* were prepared by concentrating 100 ml. of a 48 hour culture by moderate centrifugation, washing three times by centrifugation, homogenizing the packed cells in a micro-homogenizer (of the type described by Potter and Elvehjem, 1936) for a period of three minutes, and suspending the homogenate in distilled water. Microscopic examination revealed that no intact cells remained after this treatment. The chemicals used in this study were obtained from commercial sources. Inorganic ortho-phosphorus was determined by the method of Fiske and SubbaRow (1925). Other details of methods will be discussed in appropriate context.

Received for publication, September 30, 1953.

TABLE 1.—*Hexokinase activity*

Reaction Mixture	Inorganic P (after hydrolysis) µg		Inorganic P (before hydrolysis) µg		P transfered µg	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Complete	57.6	67.2	31.4	32.8	23.4	13.4
Without Mg	90.0	90.0	27.4	28.8	0	0
Without ATP	4.0*	4.0*	4.0*	4.0*	0	0
Without enzyme	83.0	88.0	17.6	15.6	0	0
Complete (time = 0)	90.0	90.0	22.4	23.4	...	...
Complete	58.6	...	27.4	...	26.4	...
Complete (2 × Mg)	53.0	...	30.4	...	29.0	...

* Accuracy of P determinations below 4 µg is questionable.
Expt. 1 Strain 5A 119×10^6 organisms in 6.0 ml.
Expt. 2 Strain 2A 75×10^6 organisms in 6.0 ml.

RESULTS

a. *Hexokinase.* The determination of hexokinase depends upon the formation of glucose-6-phosphate in the test system:

Glucose + ATP → Glucose-6-phosphate + ADP

Glucose-6-phosphate is characterized by its resistance to 7-minute hydrolysis in 1N HCl at 100° C.

The conditions of assay were as follows: a complete reaction mixture had ATP 0.003M, $MgSO_4$ 0.004M, Dextrose 1%, enzyme preparation 1.0 ml. and Tris Buffer 0.04M at pH 7.4, in a total volume of 3.0 ml. The reaction was incubated at 37.7° C. for 30 minutes. The reaction was terminated by the addition of 1.0 ml. of 5% trichloracetic acid. An aliquot was removed at once, and a determination was made of the inorganic ortho-phosphorus and inorganic ortho-phosphorus released by 7-minute hydrolysis in 1N HCl at 100° C.

The data given in Table 1 indicate that hexokinase activity was present in homogenate preparations of *Trichomonas vaginalis.*

b. *Aldolase.* Aldolase activity determination was dependent upon the formation of triose phosphate in the reaction: Fructose-1,6-diphosphate (HDP) → 3-phosphoglyceraldehyde + Dihydroxyacetone phosphate. Assay for this enzyme was based upon the fact that the products of the reaction differed from other phosphorylated esters in that the phosphate was quite readily hydrolyzed by 1N NaOH at room temperature in 20 minutes, as shown by Meyerhof and Lohmann (1934).

Cyanide was used as a triose phosphate binding reagent, which prevents further enzymatic triose degradation, as shown by Speck and Evans (1945), and Read (1951).

TABLE 2.—*Aldolase activity*

Reaction Mixture	Inorganic P (after hydrolysis) µg		Inorganic P (before hydrolysis) µg		Alkali-labile P formed µg	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Complete	76.4	116.0	15.8	38.0	22.8	18.0
Without KCN	82.4	100.8	16.4	35.2	28.2	5.6
Complete (time = 0)	27.8	60.0	8.8	30.0	...	...
Without HDP	4.0*	4.0*	4.0*	4.0*	0	0
Without enzyme	22.8	50.4	4.0*	23.6	0	0

* Accuracy of P determinations below 4 µg is questionable.
Expt. 1 Strain 2A 131.4×10^6 organisms in 4.5 ml.
Expt. 2 Strain 5A 84.3×10^6 organisms in 4.5 ml.

Assay was carried out as follows: the complete reaction mixture contained HDP 0.033M, KCN 0.250M, enzyme preparation 1.0 ml., Tris Buffer 0.04M at pH 7.4, in a total volume of 3.0 ml. The reaction mixture was incubated at 37.7° C. for 20 minutes. The reaction was terminated by the addition of 1.0 ml. of 5% trichloracetic acid. An aliquot was removed at once, and a determination was made of the inorganic ortho-phosphorus and the inorganic ortho-phosphorus released by 20 minute hydrolysis in 1N NaOH at room temperature. The data given in Table 2 indicate that the homogenate preparation possessed aldolase activity.

DISCUSSION

This initial study dealing with the intermediary metabolism of *Trichomonas vaginalis* furnishes evidence that the preliminary stages of carbohydrate utilization have the same characteristics as do those of vertebrate tissue and yeast. This study is the first to indicate that the parasitic flagellates of warm blooded vertebrates found outside of the blood stream also possess a phosphorylative mechanism for utilization of carbohydrate. Further experiments dealing with the anaerobic utilization of carbohydrates strengthen this proposal. They will be reported at a later date.

REFERENCES

FISKE, C. H. AND SUBBAROW, Y. 1925 The colorimetric determination of phosphorus. J. Biol. Chem. **66**: 375–400.

JOHNSON, G. AND TRUSSELL, R. E. 1943 Experimental basis for chemotherapy of *Trichomonas vaginalis*. Infestations I. Proc. Soc. Exptl. Biol. and Med. **54**: 245–249.

MEYERHOF, O. AND LOHMANN, K. 1934 Über die enzymatische Gleichgewichtsreaktion zwischen Hexosediphosphorsäure und Dioxyacetonphosphorsäure. Biochem. Ztschr. **271**: 89.

NINOMIYA, H. AND SUZUOKI, Z. 1952 The metabolism of *Trichomonas vaginalis* with comparative aspects of trichomonads. J. Biochem., Japan, **39** (4) : 321–331.

POTTER, V. R. AND ELVEHJEM, C. A. 1936 A modified method for the study of tissue oxidations. J. Biol. Chem. **114**: 495–504.

READ, C. P. 1951 Studies on the enzymes and intermediate products of carbohydrate degradation in the cestode *Hymenolepis diminuta*. Exper. Parasit. **1** (1) : 1–18.

SPECK, J. F. AND EVANS, E. A., JR. 1945 The biochemistry of the malaria parasite. II. Glycolysis in cell-free preparations of the malaria parasite. J. Biol. Chem. **159**: 71–81.

TRUSSELL, R. E. AND JOHNSON, G. 1941 Physiology of pure culture of *Trichomonas vaginalis*. III. Fermentation of carbohydrates and related compounds. Proc. Soc. Exptl. Biol. and Med. **47**: 176–178.

RESEARCH NOTES

AN UNSUCCESSFUL ATTEMPT TO ESTABLISH *EUSTRONGYLIDES* IN THE BLACK-CROWNED NIGHT HERON, *NYCTICORAX NYCTICORAX HOACTLI*

The reported definitive hosts of *Eustrongylides ignotus* are *Ardea coco* and *Botaurus pinnatus* (Jaegerskiold, 1909, Nova Acta Regiae Soc. Sci. Upsal. ser. IV, **2**: 1–48), *Ardea herodias* (Chapin, 1926, J. Parasit. **13**: 86–87) and *Nycticorax nycticorax hoactli* (Cram, 1934, J. Parasit. **20**: 71). Larval stages have been reported to occur naturally in bullfrogs (Von Brand, 1944, Proc. Helm. Soc. Wash. **11**: 23–27) and have been consistently observed by a number of workers (Chapin, 1926; Hunter, 1937, Suppl. 26th Ann. Rep. New York State Conserv. Dept. Biol. Surv. No. 11: 264–273; Hunter, 1942, Bull. 63, Conn. Geol. Nat. Hist. Surv., 228–288; Cullinan, 1945, J. Parasit. **31**: 109–112; and others) from various fishes. In the Washington-Baltimore area we have found a high incidence of infection in the bull-head minnow, *Fundulus diaphanus*, as suggested by Chapin (1926) ; well over half of several hundred minnows examined during the summer of 1951 revealed 1 to 14 larval *Eustrongylides* encysted in the mesenteries. An incidence of less than 10% was observed in *Gambusia affinis*, which apparently constitutes a new host record for *Eustrongylides*. These larvae were tentatively identified as *Eustrongylides ignotus* since this is the only adult species observed in herons in this region, and since a larval

form isolated from *Fundulus heteroclitus* and maintained *in vitro* by Von Brand and Simpson (1944, J. Parasit. **30:** 121–129) molted into an adult male *Eustrongylides ignotus.*

In an investigation of the life cycle of this nematode, it was desirable to have a ready supply of *Eustrongylides* eggs for use in attempting to infect possible primary intermediate hosts. Consequently, a young female black-crowned night heron was procured from the National Zoological Park, Washington, D. C., through the courtesy of Mr. Herbert S. Davis. This bird had been reared in captivity; repeated examinations of the droppings by direct smear and $ZnSO_4$ flotation techniques revealed no helminths or eggs.

Over a period of 52 days the bird was fed 14 *Eustrongylides* larvae, 8 from *Fundulus diaphanus* and 6 from *Gambusia affinis.* The minnows were obtained from the Kenilworth Aquatic Gardens, Washington, D. C. The worms were dissected free of the viscera and in most cases they remained intact in the tough, fibrous cyst surrounding them; inadvertently, however, the cyst was ruptured in 2 dissections and in these cases the anterior end of the worm had freed itself from the cyst. The worms were placed in the esophagus with forceps and the bird was induced to swallow. The cage was examined for several hours following administration of the worms to make certain that they had not been vomited, since vomiting was observed occasionally following feeding.

The number of worms fed (14) was considered to be sufficient to insure getting both males and females and yet not enough to debilitate the bird, should they develop to maturity. The prolonged feeding period (52 days) would probably correspond to the way in which the bird would be exposed to helminths in nature.

The bird was fed commercial horse meat with occasional supplements of eviscerated fish. Daily fecal examinations were made for the presence of helminths and eggs for the first month following administration of the worms, then at approximately weekly intervals for 35 weeks. Since all of these examinations were negative, it was decided to sacrifice the bird to determine whether the worms had established themselves.

At necropsy one male *Contracaecum spiculigerum* was found in the mid-region of the small intestine. Presumably this nematode was present at the beginning of the experiment, since its acquisition from the food given the bird is unlikely.

One is at a loss to explain the failure of the nematodes to establish themselves. It hardly seems possible that immunological factors could be responsible since the heron used was a young animal and relatively free of helminths. Evidence that it is the final larval stage which occurs in minnows is strong since a larva therefrom was shown to reach maturity when maintained *in vitro* (see above). That the infection would be acquired in some manner other than through the ingestion of infected fish seems unlikely since fish constitute the principal food of herons.

The author is indebted to Dr. Ernest A. Lachner of the U. S. National Museum, Washington, D. C., for identification of the minnows.—John R. Elsea, *The Catholic University of America, Washington, D. C.*

A SEAL HOST OF THE ACANTHOCEPHALAN *CORYNOSOMA:* CORRECTION

Van Cleave (1953a, J. Parasit. **39:** 1–13; 1953b, Ill. Biol. Mon. **23** [1, 2]: 1–179) reported three acanthocephalan species of the genus *Corynosoma* (Palaeacanthocephala) from the "gray seal (*Halichoerus grypus*)." These included *Corynosoma strumosum* (Rudolphi, 1802), *C. hadweni* Van Cleave, 1953 and *C. falcatum* Van Cleave, 1953. *Corynosoma strumosum* and *C. hadweni* occur in a variety of hosts (Van Cleave, 1953b). *Corynosoma hadweni* and *C. falcatum* were described as new species (Van Cleave, 1953a). Van Cleave (1953a, b) noted that the parasites from *Halichoerus grypus* were collected by the late Dr. Seymour Hadwen at Unalaska on December 4, 1920 (VC 1932, VC 2637) and January 21, 1921 (VC 1933), listing the indicated serial numbers from his "accession book."

The collection of specimens of *Halichoerus grypus* from the Bering Sea would constitute a very unusual and important distribution record for that seal. Dr. V. B. Scheffer, in written communication to Dr. H. W. Stunkard (then Editor, Journal of Parasitology) strongly questioned the validity of this host identification. He noted that, according to Dunbar (1949, Fish. Res. Bd. Canada, Bull. **85:** 1–22), "*Halichoerus grypus* 'is a North Atlantic species of a very limited range, and only very occasionally enters arctic or subarctic waters. It is rare at the level of Belle Isle on the American side.'"

The personal correspondence which accompanied the Hadwen collection is not available in Van Cleave's files. Items VC 1932, VC 1933 and VC 2637 in the latter's accession book were examined closely. The host, in all cases, is listed merely as "Gray seal." The locality of VC 1932 (dittoed for VC 1933) is given as "Unalaska," that of VC 2637 as "Unalakleu Alas." Penciled notations, apparently added to the original records, state that VC 1932 is the "same as 2637" and that VC 2637 is the "same as 1932."

Hadwen (1922, J. Am. Vet. Ass. 61, n.s., **14**: 374–382) specifically identified seals taken at "Unalakleet, Alaska . . . in December, 1920," in the course of his investigations there, as *Phoca richardi*. He further stated that "the seals harbored a large number of a species of Acanthocephala." He did not identify the latter.

The notation by Van Cleave in his accession book that VC 1932 and VC 2637 are the same must refer to aggregations of specimens from the same general collection. It cannot refer to individuals, as both VC 1932 and VC 2637 contained both *C. strumosum* and *C. hadweni*, but only VC 2637 contained specimens identified as *C. falcatum* (Van Cleave, 1953a, b). "Unalakleu Alas." must indicate Unalakleet, Alaska. Thus the choice of two localities was offered in the notes. It appears unlikely, although not impossible, that Hadwen collected seals at Unalaska (in the Aleutian chain) on December 4, 1920 and January 21, 1921 and also collected extensively (reindeer and seals: Hadwen, 1922) at Unalakleet, Alaska (on Norton Bay) "in December 1920." It is thus most probable that Van Cleave (1953a, b) selected the incorrect locality designation (Unalaska) for the collections of Acanthocephala. The probable sources of the error must have been the inability to decipher correctly the handscript (ordinarily cramped) on the labels which accompanied the specimens, the lack of complete data in the accompanying correspondence and, finally, the decease of the collector (Hadwen).

The accession book data are strong evidence that the seal hosts were identified to Van Cleave as "gray seals" only. His designation of *Halichoerus grypus* is correct for that species. *Phoca richardi* is commonly called the "gray harbor seal." Its designation as "gray seal" is doubtless common among individuals in Alaska who are unaware of the true "gray seal" of the North Atlantic. Hadwen, primarily a parasitologist, doubtless used the local common name but was aware of the correct scientific designation of these seals.

In view of the above data and interpretations, the author believes that the host designations for *Corynosoma strumosum, C. hadweni,* and *C. falcatum* should be amended to include *Phoca vitulina richardi* (see Doutt, 1942, Ann. Car. Mus. **29**: 61–125) in place of *Halichoerus grypus* and that the locality of the collection should be changed to Unalakleet, Alaska. The speciation of *C. hadweni* and *C. falcatum* was based entirely on morphological characters and is in no way affected by the suggested changes.—Francis J. Kruidenier, *University of Illinois.*

PARASITES FOUND IN THE SEA LAMPREY, *PETROMYZON MARINUS*, FROM LAKE MICHIGAN

During the years, 1951 and 1952, 76 mature sea lampreys were examined for parasites. Twelve of these were active feeders taken in February from nets set in Lake Michigan near Sturgeon Bay, Wis. Sixty-five were upstream migrants taken in June from U. S. Fish and Wildlife Service traps in Hibbards Creek and the Kewaunee River, tributaries of Lake Michigan in Door and Kewaunee Counties, Wis., respectively. All were examined while fresh and the parasites living.

Five of the 12 actively feeding lampreys and 36 of the 65 upstream migrants were infected with helminth parasites. Thirty-two of the infected lampreys had helminths of only 1 species while 9 had 2 species. No more than 2 species of helminths were found in 1 host. All were confined to the alimentary canal. The only species of protozoan parasite, a species of *Trichodina*, was found on the gills of 1 actively feeding lamprey. No pathological disturbances were associated with any of the parasites. There appeared to be no difference in the susceptibility of the sexes since 22 of 39 male and 19 of 37 female lampreys were infected.

The parasite most frequently encountered was *Proteocephalus exiguus* LaRue. Plerocercoids or young specimens of this worm, few of which were mature enough to have eggs, were found in 35 lampreys. Their numbers varied from 1 to 49 with an average of 7.2 per host. One larval *Cystidicola stigmatura* Ward and Magath, which probably had been ingested from the swim bladder of a coregonid fish, and one *Neoechinorhynchus cylindratus* Van Cleave, were found. This is the first report of these parasites from the lamprey. Also 10 *Echinorhynchus coregoni* Linkins, 4 *Echinorhynchus leidyi* Van Cleave, 2 immature *Abothrium* sp., and 2 immature *Triaenophorous crassus* Forel were found in 7, 4, 2, and 2 lampreys respectively. These species had been reported previously by Applegate (1950; U. S. Dept. of Int., Fish and Wildlife Service. Special Sci. Rep.: Fisheries No. 55) and by McLain (1952; Trans. Amer. Fisheries Soc., **81**: 94–100) in sea lampreys from Lake Huron and its tributaries.

Thanks are due to Mr. Leonard Joeris of the U. S. Fish and Wildlife Service, Sturgeon Bay, Wis., for help in procuring lampreys, and to Dr. George R. LaRue, Zoological Division, Bureau of Animal Industry, Beltsville, Md., for helping to identify the specimens of *Proteocephalus exiguus*.—Harry G. Guilford, *University of Wisconsin Extension Center, Green Bay, Wisconsin.*

INTESTINAL MYIASIS IN THE SYRIAN HAMSTER

Fifty Syrian hamsters were purchased by this laboratory from a local dealer on September 23, 1953. On October 6, one of these animals, which had appeared sluggish and lethargic for 2 or 3 days, was found dead in its cage. Autopsy revealed 19 living maggots (10–13 mm. long) established in the rectum causing complete stasis, intestinal hemorrhage and marked flatulence. Examination of these maggots revealed a pointed anterior end, blunt posterior end and the characteristic spiracles and cephalo-pharyngeal skeleton of *Musca domestica* larvae. Seven of these maggots were preserved and the remainder placed in a container at room temperature where they developed into pupae on October 9. Emergence of 4 flies occurred 6 days later and 6 more flies made their appearance from the pupa stage on the following day, October 16. These flies were identified as the common housefly, *M. domestica*, by both senior and junior authors.

Five of these reared flies (1 male and 4 females) and 1 hamster were then placed in each of 2 fly proof containers for further observation. These 2 hamsters were killed and examined, 1 on November 10, and the other on November 26. No sign of myiasis was found. None of the other 49 hamsters obtained from the original source exhibited any signs of intestinal myiasis. The infection here reported might have originated in either of two ways: 1. the hamster ingested food contaminated by fly eggs or larvae and the larvae succeeded in reaching the rectum alive, or 2. fly eggs were deposited in close proximity to, or association with, the hamster and the larvae entered the anus. Further experimentation is planned to determine method of infection of the hamster by these maggots.

This finding is reported as part of a study supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-495.—Wayne W. Wantland and E. P. Lichtenstein, *Division of Science, Illinois Wesleyan University, Bloomington, Illinois.*

DELPHINAPTERUS LEUCAS, A NEW HOST RECORD FOR THE WHALE-LOUSE, *PARACYAMUS NODOSUS* (LÜTKEN)

Recently, through the courtesy of Dr. Torben Wolff, a collection of whale-lice (Amphipoda: Cyamidae) was received for identification from the University Zoological Museum of Copenhagen. Among the specimens was a vial containing about 50 individuals of a species determined by the author as *Paracyamus nodosus* (Lütken, 1860) Stephensen, 1942. The accompanying label gave the host as the beluga (*Delphinapterus leucas*) and the locality as Greenland. The label carried no further data. Prior to this report, *P. nodosus* was known only from the narwhale, *Monodon monoceros* (Lütken, 1873, Vidensk, Selsk. Skr. S5, **10:** 231–284; Stephensen, 1942, Tromsø Mus. Skr. **3:** 443–456).

Lütken (1860, Forh. Skandinav. Naturf. ottende Møde, 590–592. Copenhagen) pointed out that prior to his naming of the species, it was known in the Copenhagen Museum as *Cyamus Belugae*. He explained that the specific name *Belugae* was misleading, since it suggested that the host was the white whale (beluga). Actually, the host was the narwhale. This error arose from the habit, by Greenlanders, of using the word "Hvidfisk," which means white whale, for both white whales and narwhales. Since the host was truly the narwhale, Lütken replaced the trivial name *Belugae* by *nodosus*, to avoid any confusion with reference to the host. The specific name *nodosus* must now stand as valid, since the name *Cyamus Belugae* had never been published.

According to Lütken (1873) and Stephensen (1942) *P. nodosus* is frequently accompanied on the narwhale by another species, *Cyamus monodontis* Lütken, 1870, which Lütken (1893, Vidensk. Selsk. Skr. S6, **7:** 421–434 also has found once on the beluga.

These are the only 2 records of whale-lice on the beluga.

It can be stated generally that whale-lice are extremely host specific. Since at no period in the life history of a cyamid is there a free swimming stage, transmission of the parasites involves direct contact of the host animals. This, of course, is accomplished within a species. But, do individuals of different cetacean species come into sufficiently intimate contact to permit transfer of the cyamids? The 2 records of parasitization of the beluga by whale-lice normally found on the narwhale, suggest that these 2 whale species may sometimes come into direct contact with one another. Both records are from areas co-inhabited by the beluga and the narwhale.—Leo Margolis, *Pacific Biological Station, Nanaimo, B. C., Canada.*

BIOLOGICAL ABSTRACTS

Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in five low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section C—Abstracts of Microbiology, Immunology, and Parasitology—affords a very complete coverage of the Parasitologists' literature. The price is only $6.00 a year (Foreign, $6.50) including the comprehensive index issue of the complete edition.

Write for a sample copy.

BIOLOGICAL ABSTRACTS

University of Pennsylvania
Philadelphia 4, Pa.

THE JOURNAL OF PROTOZOOLOGY

The Society of Protozoology announces a new *Journal of Protozoology,* to begin publication in the spring of 1954. Papers concerned with original work on any aspect of the study of Protozoa may be submitted to the Editor, William Trager, The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York 21, N. Y.

The Journal will consist of 4 issues per year. The annual subscription will be $9 for all except graduate students, for whom it will be $6. Inquiries regarding subscriptions and membership in the Society of Protozoologists should be addressed to Norman D. Levine, Secretary, College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

Publications of American Veterinary Medical Association

Journal of the American Veterinary Medical Association
(Monthly)

Subscription price
$10.00 a year
Canada—$10.50
Foreign—$10.50

American Journal of Veterinary Research
(Quarterly)

Subscription price
To members of AVMA—$2.50

To non-members—$5.00
Canada—$5.30
Foreign—$5.30

Proceedings Book of the AVMA
Free to members
Non-members, $5.00

SCANNED 15 of 43
journal-of-parasitology_1954-08_40_4
2025-06-08
Box: IA-CA-200003202
Old Pallet: IA-CA-2000032
The Journal of Parasitology 1954-08: Vol 40 Iss 4
INTERNET ARCHIVE
0022-3395

asitology

Number 4

RED SEA.
IDS AND

basia, Cairo.
of Nebraska.

her contribution to the
ontributions by me are
collected by the author
of Nebraska during the

, Faculty of Medicine,

r examination. These
enoides vittatus locally
called "Enber baladi," from Ghardaga and Koseir, and from 5 *Mulloides auriflamma* locally called "Enber" from Ghardaga.

Description: Body narrow, elongated and almost cylindrical, with more or less parallel margins, slightly converging in anterior quarter of body. Preacetabular portion quite short, distinctly narrower than remainder of body in unpressed specimens. Body rounded posteriorly. Length 1.052 to 4.114 mm. by 0.273 to 0.595 mm. in maximum breadth. Acetabulum larger than oral sucker, located about one-ninth of body length from anterior end, slightly pedunculated, being borne on short broad stalk. Acetabulum complicated, consisting of usual muscular cup, 0.147 to 0.315 mm. in diameter, and 12 processes each having an outer covering of body cuticula and core from musculature of sucker. Eight acetabular processes located peripherally and arranged in somewhat separated pairs, one pair extending from each antero- and each posterolateral sector of acetabulum. Each pair originating from short thick common trunk, divided distally into 2 processes, gradually tapering toward their tips. Four remaining processes digitiform, located nearer sucker opening, 2 originating anterior and 2 posterior to sucker opening which they may close when interlocked. Median processes in 1 specimen 0.042 to 0.063 mm. long, peripheral processes 0.084 to 0.105 mm. Papillae sometimes retracted and difficult to observe. Cuticle smooth and unarmed.

Oral sucker anterior, subterminal and ventral, measuring 0.082 to 0.147 mm. in diameter. Prepharynx very short, pharynx muscular, well-developed, spindle-shaped, 0.105 to 0.168 mm. long by 0.063 to 0.126 mm. wide. Esophagus of medium length, intestinal forking anterior to ventral sucker. Crura simple, laterally situated, and uniting near posterior end of body to form an arc connected to outside by short tube, opening independently through sub-terminal ventral anal pore.

Testes 2, in tandem, indented, situated in middle third of body between crura. Indentations variable, sometimes one or both testes very little indented. Testes subequal, 0.147 to 0.35 mm. in diameter, separated by variable distances in different specimens, equalling width of testes

Received for publication, December 18, 1953.
* Studies from the Department of Zoology, University of Nebraska, No. 271.

or much less. Vesicula seminalis externa comparatively long and tortuous, measuring about one quarter total length of worm, beginning posteriorly between crura about mid-way between ventral sucker and ovary, and proceeding anteriorly, meanwhile becoming more slender. Cirrus sac indistinct, situated usually toward left side of body at level of intestinal forking.

Ovary typically quadrilobate, pretesticular, in median plane between intestinal crura, and in anterior part of middle third of body. Ovary 0.063 to 0.21 mm. in its short obliquely directed antero-posterior dimension, and 0.109 to 0.231 in its longer oblique transverse dimension. Mehlis' gland preovarian. Ovary separated from anterior testis by width of ovary or less.

Vitelline glands profusely developed, follicles small and irregularly shaped, extending from ovarian region to posterior end of body. In gonadal region follicles occurring lateral to gonads, coming more closely together medially posterior to and between testes, tending to be arranged in 4 distinct longitudinal bands, 2 on each side more or less separated by intestinal crura.

Uterus preovarian, with transverse convolutions, between crura, opening into common genital atrium anterior to ventral sucker and lateral to median plane. Eggs comparatively few, large, ovoid, light golden yellowish, operculate and with knob-like thickening at other pole. Eggs 0.05 to 0.063 mm. by 0.029 to 0.042 mm.

Excretory vesicle elongated tubular, with parallel sides, reaching anteriorly to ovary, and lying dorsal to gonads and to posterior arch of intestinal crura.

Discussion: This species closely resembles *O. elongatus* Ozaki, 1928, but differs from that species in the presence of 2 pairs of median digitiform papillae on the acetabulum and also in the shape of the peripheral papillae.

Opecoelus upenoidis n. sp.
Figure 4

Nine specimens of this species were obtained from the alimentary tracts of 6 *Upenoides vittatus* locally called "Enber baladi" from Ghardaga. This species occurred in a mixed infection with *O. adelongatus* and was first looked upon as an aberrant form of that trematode. It was thought advisable, however, to separate the 2 forms as the differences in morphology, especially the shape of the ovary and testes, which are here entire, warrant this separation. The name *O. upenoidis* is suggested for this form.

Description: Trematodes spatulate, tapering gradually towards oral sucker, rounded posteriorly, length 1.424 to 2.398 mm. by 0.131 to 0.315 mm. in maximum breadth at level of testes. Cuticle lacking scales or spines. Acetabulum larger than oral sucker, its width often exceeding its length of 0.108 to 0.158 mm. Acetabulum set on short stalk, and provided with 4 pairs of reduced peripheral papillae and 2 pairs median.

Digestive system similar to that of *O. adelongatus.* Oral sucker 0.088 to 0.106 mm. in diameter, pharynx muscular, 0.06 to 0.088 mm. long by 0.042 to 0.07 mm. broad.

Testes 2, tandem, almost entire, separated by short space, intercrural and situated in third quarter of body, slightly more posterior than in *O. adelongatus.* Testes subequal, 0.095 to 0.147 mm. in diameter. Vesicula seminalis externa long, slightly tortuous, and extending about two thirds distance between posterior border of acetabulum and anterior border of ovary. Cirrus sac and genital opening poorly developed and lateral to esophagus.

Ovary almost entire, crescentic or triangular, sometimes provided with small indistinct lobe, and situated at posterior-most part of anterior half of body. Diameter of ovary 0.063 to 0.088 mm. Vitelline follicles distributed along intestinal crura posterior to ovary and less profusely developed than in *O. adelongatus.* Eggs few, ovoid with smooth shells, measuring 0.053 to 0.063 by 0.028 by 0.046 mm.

Discussion: This species resembles closely *O. adelongatus* from which it differs in having almost entire gonads, testes situated further posterior, and vitelline forlicles less profusely developed.

Opecoelus thapari n. sp.
Figure 5

The description of this species is based upon 8 stained and mounted specimens obtained from the alimentary tracts of 6 *Upenoides vittatus* locally called *"Enber baladi"* from Ghardaga.

Description: Trematodes 0.998 to 2.34 mm. in length by 0.176 to 0.318 mm. in maximum breadth near ventral sucker, sides almost parallel, and posterior end rounded. Ventral sucker larger than oral, situated at posterior border of first fifth of body, borne on short broad pedicle. Ventral sucker 0.158 to 0.189 mm. long by 0.158 to 0.265 mm. broad, breadth usually exceeding length. Processes of acetabulum, so characteristic of genus *Opecoelus* very rudimentary and in most specimens discernible with difficulty, only 8 peripheral papillae discernible and these arranged in 4 pairs, 2 antero-lateral and 2 postero-lateral.

Opening of oral sucker directed ventrally. Oral sucker 0.066 to 0.138 mm. in diameter, smaller than ventral sucker. Pharynx muscular, well-developed, partially overlapping oral sucker dorsally. Pharynx 0.044 to 0.127 mm. long by 0.04 to 0.074 mm. broad. Esophagus of medium length, divided into 2 simple intestinal branches anterior to ventral sucker. Intestinal crura situated laterally and united to form an arch at posterior end of body, discharging through a simple short canal leading to outside at posteriorly situated anal opening.

Testes 2, smooth, ovoid or spheroid, of about same size, and situated in tandem in posterior half of body in mid-plane between intestinal crura. Anterior testis situated just posterior to equator of body and measuring 0.119 to 0.223 mm. long by 0.11 to 0.167 mm. broad. Distance between testes about equalling diameter of posterior testis. Latter 0.123 to 0.254 mm. long by 0.11 to 0.154 mm. broad. Vesicula seminalis externa long, but not tortuous. Its broad posterior end lies about midway between ovary and posterior border of ventral sucker in space between crura. Gradually narrowing toward its anterior extremity it joins the small cirrus sac just anterior to intestinal bifurcation. Cirrus sac small, ovoid, at left of esophagus, opening into genital pore near posterior border of oral sucker and near left side of trematode.

Ovary spheroidal, entire, 0.062 to 0.101 mm. in diameter, pretesticular, median and slightly pre-equatorial. Mehlis' gland near and anterior to ovary. Vitelline glands well-developed, distributed between posterior end of body to posterior border of acetabulum, but vitelline fields interrupted by gonads, thus forming 4 groups. Posterior to hind testis vitelline follicles forming an uninterrupted mass mainly between intestinal crura which may be overlapped by a few follicles dorsally or ventrally. Anterior to posterior testis follicles becoming distinctly massed into 2 groups, mainly between intestinal crura with a median space free from follicles.

Uterus preovarian, occupying intercrural space, and opening into common genital atrium, lateral to esophagus. Eggs few, large, ovoid, 0.066 to 0.07 mm. by 0.035 to 0.048 mm., operculate and with slight thickening of shell at other pole.

Discussion: This species is closest to *O. adsphaericus* Manter and Van Cleave, 1951, from which it can be differentiated by its smaller size, its acetabulum with 4 pairs of papillae instead of 3, vesicula seminalis not sinuous and longer, ovary entire not trilobed, eggs large and fewer.

I have pleasure in naming this species after Professor G. S. Thapar of Lucknow, India.

Family: Mesometridae

Arthurloossia loossi n. g., n. sp.

Figures 6–7

Nineteen specimens were stained and mounted, 5 of which were fully mature and the others were in a less developed state. They were obtained from *Pseudoscarus harid,* locally called "Wariga" or "Harid" and from *Teuthis oramen,* locally called "Sigan," from Ghardaga.

Description: Mature specimens fairly large, 6.278 to 8.17 mm. in length and 1.63 to 2.15 mm. in maximum breadth, flattened dorso-ventrally with more or less parallel sides, slightly tapered anteriorly in some specimens, but in others, especially young ones, smoothly rounded. Posterior extremity rounded, but possessing a median knob-like protuberance. Cuticle provided with minute spines, especially anteriorly. Ventral sucker lacking. Oral sucker spheroid, 0.264 to 0.29 mm. long by 0.273 to 0.326 mm. broad, deeply imbedded in parenchyma of anterior body extremity, and connected to mouth by short narrow vestibule. Esophagus long, equalling about one eighth to one sixth length of worm. Thickened walls of its posterior part simulating a poorly developed pharynx, distinguishable only in young immature worms. Intestinal crura simple, broad, slightly undulating and extending only to testes.

Testes two, obliquely situated in posterior part of body, elongated, sub-equal, 1.080 to 1.554 mm. long by 0.79 to 0.925 mm. wide. Vasa efferentia uniting to form vas deferens near

junction of second and third thirds of body. Vas deferens dilated near equator of body, forming long tortuous vesicula seminalis, latter narrowing to insignificant genital atrium opening mid-ventrally near mid-esophageal level. Cirrus and cirrus sac lacking; terminal part of male duct surrounded merely by cluster of unicellular glands.

Ovary spheroid, 0.413 to 0.616 mm. in diameter, posterior to and between testes and in plane more ventral to these. Mehlis' gland well-developed, pyriform, posterior and lateral to ovary. Uterine canal passing between testes, then forming convolutions between intestinal crura and opening into simple atrium together with male duct. Uteri of mature worms filled with thin-shelled, colorless ovoid eggs. Latter with operculum at narrow pole and lacking knobs or filaments. Eggs 0.086 to 0.09 by 0.054 to 0.062 mm.

Vitelline glands well-developed, prolific and in form of large irregularly shaped follicles extending forward from anterior border of testes to bifurcation of intestinal crura, and spreading medially as well as laterally to crura, and arranging themselves roughly in 4 longitudinal rows. Common vitelline ducts passing posteriorly between testes to reach oviduct.

Excretory system composed of at least 4 major longitudinal vessels extending backward from intestinal bifurcation to anterior border of testes. Two vessels of 1 side uniting to form a narrow duct on either side of posterior end of body. These 2 main ducts uniting at posterior level of ovary to form single short duct opening to outside terminally and posteriorly through excretory pore. Major longitudinal ducts intercommunicating and receiving others from anterior extremity of body.

Discussion: To the family Mesometridae Poche, 1926, have been assigned three genera: *Mesometra* and *Centroderma* Lühe, 1901 (this genus was considered by Lühe [1901] to be a subgenus of *Mesometra,* but it was raised to generic rank by Poche [1926]) and the genus *Wardula* Poche, 1926. The present paper adds a fourth genus, named for the eminent helminthologist, the late Arthur Looss.

TABLE 1.—*Main differences between the genera of the family* MESOMETRIDAE *Poche, 1926*

	Mesometra	*Wardula*	*Centroderma*	*Arthurloossia*
Shape of body ...	discoidal and rounded	cylindroid and elongated, length 5–7 times breadth	oval, elongated (length about 3 times breadth)	elongated (length about 3 times breadth)
Oral sucker	subterminal, superficial, opening ventrally	?	subterminal, superficial, opening ventrally	deep in parenchyma, opening through vestibule to surface anteriorly
Pharynx	poorly developed, hardly visible	flask-shaped	poorly developed, hardly visible	poorly developed, hardly visible
Testes	oval, elongated, opposite	oval,broad, close together, tandem	globular,opposite	elongate, obliquely situated
Ovary	median, lobed, broader than long	median, oval entire,broader than long	median, oval, entire, broader than long	median, spheroid, entire
Vitellaria	prolific, follicles small, occupying greater part of body, extending posterior to testes	prolific, irregular and wide-meshed, net-like, branched, occupying most of body	prolific, follicles small, extending from bifurcation of ceca to testes	prolific, follicles large, irregularly shaped, extending from intestinal bifurcation to testes
Eggs	75 by 35 microns, presumably without filament	without filament	155 by 45 microns, with long filament, measuring 175 microns long	88 by 58 microns, filament and knob lacking

The genus *Arthurloossia* is nearest to *Centroderma;* the differences between it and the other genera are tabulated in table 1.

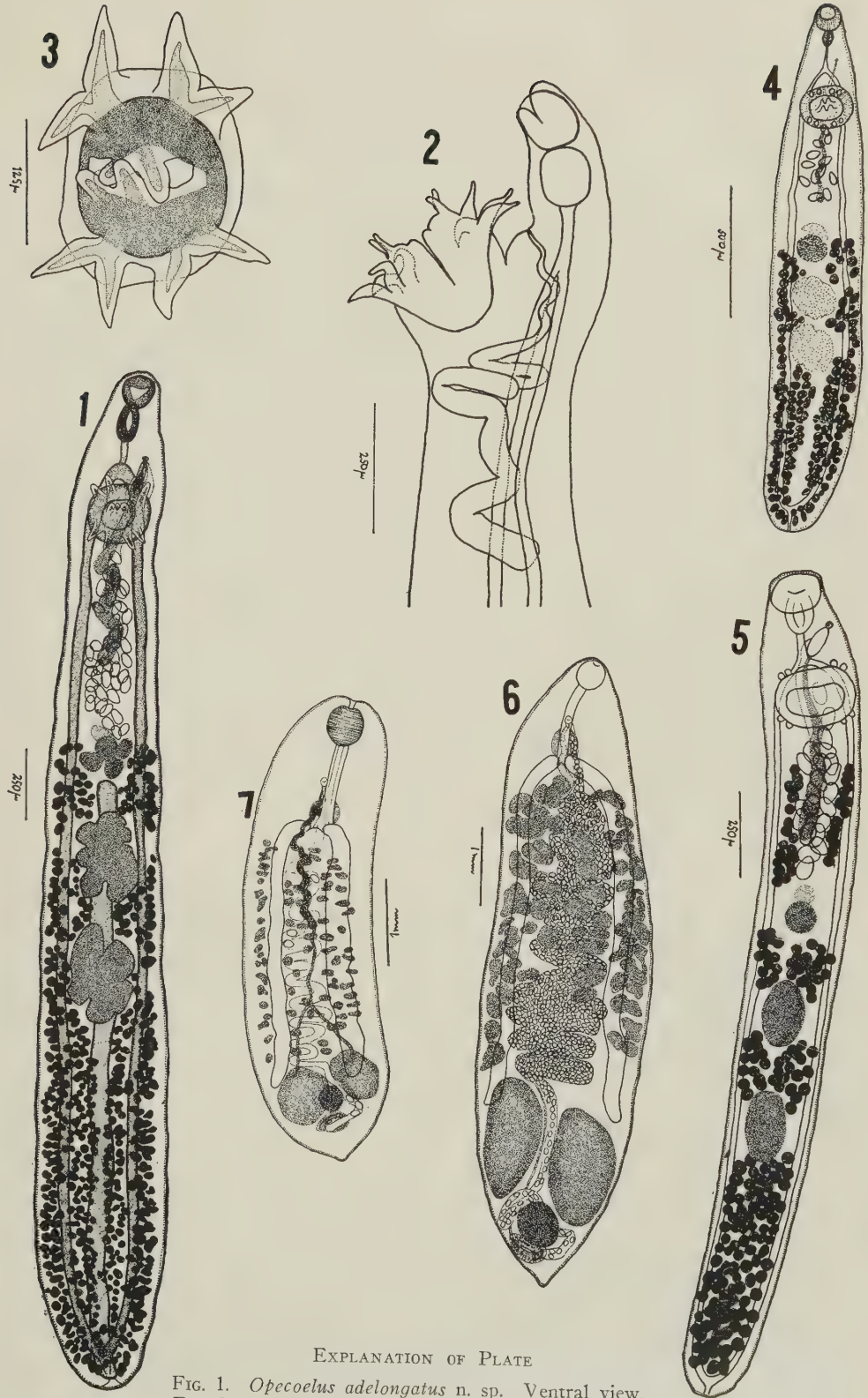
REFERENCES

LÜHE, M. 1901 Ueber *Monostomum orbiculare.* Centrbl. Bakt., 1 Abt., **29**: 49–60.

MANTER, H. W. AND VAN CLEAVE, H. J. 1951 Some digenetic trematodes, including eight new species, from marine fishes of LaJolla, Calif. Proc. U. S. Nat. Mus. **101**: 315–340.

OZAKI, Y. 1928 On some trematodes with anus. Jap. J. Zool. **2** (1) : 5–33.

POCHE, F. 1926 Das System der Platodaria. Arch. Naturg. (1925), Abt. A, **91** (2) : 1–240, (3) 241–458.



EXPLANATION OF PLATE

FIG. 1. *Opecoelus adelongatus* n. sp. Ventral view.
FIG. 2. *O. adelongatus* n. sp. Lateral view of anterior end.
FIG. 3. *O. adelongatus* n. sp. Ventral view of acetabulum.
FIG. 4. *O. upenoidis* n. sp. Ventral view.
FIG. 5. *O. thapari* n. sp. Ventral view.
FIG. 6. *Arthurloossia loossi* n. g., n. sp. Ventral view.
FIG. 7. *Arthurloossia loossi* n. g., n. sp. Dorsal view of young form.

THREE NEW FLEAS FROM JAPAN (SIPHONAPTERA)

Everett W. Jameson, Jr. and Kôhei Sakaguti

Department of Zoology, University of California, Davis, California
and Hamawaki-cho 74, Nishinomiya, Hyogo Prefecture, Japan

The flea fauna of Japan, althought a rich and interesting one, has not been exhaustively studied; and the records and descriptions are, for the most part, scattered and incomplete. As the fleas of the Holarctic Region are being more intensively studied every year, it has become apparent that many of the species and genera are quite widespread. In order to assist in the identification of Japanese fleas, three species are here described and illustrated.

The following records help to characterize the flea fauna of Japan. The majority of papers on Japanese fleas are concerned with the species that parasitize commensal rodents, and the native fauna is poorly known. The three genera discussed here indicate that there is at least one group (*Stivalius*) from the tropical Orient. Mt. Fuji now is the northernmost point where the family (Pygiopsyllidae) occurs, and it is of interest that the species in question (*S. aestivalis*) seems to occur in the adult stage only in the summer months. The presence of *Atyphloceras* (= *Saphiopsylla*) and *Rhadinopsylla* requires no special comment; both are Holarctic in distribution. *Atyphloceras* is known from one other Old World species, but this is the first record for the genus in the Orient; several species are described from North America. Several kinds of *Rhadinopsylla* are known from the Asiatic mainland near Japan, and the genus is well represented elsewhere in the northern hemisphere.

Atyphloceras shogakii, new species
Fig. 1, A–G

We follow Hopkins (1952: 365) in synonymizing *Saphiopsylla* Jordan, 1931, with *Atyphloceras* Jordan and Rothschild, 1915. With this interpretation, there is 1 species (*nupera* Jordan) in Europe, 1 (*bishopi* Jordan) in eastern North America, and 5 (*longipalpus* Stewart, *multidentatus* Fox, *felix* Jordan, *echis* Jordan and Rothschild, and *tancitari* Traub and Johnson) in western North America. The species described here is more closely allied to *nupera* and *bishopi* in the outline and chaetotaxy of the head and the labial palpus; it is, however, very different in some other respects. Our new species is widely separated, geographically and morphologically, from other named forms of *Atyphloceras*; and would perhaps be placed in a distinct genus by some workers.

Male: Head (Fig. 1, A) with 3 rows (or 4 poorly-defined rows) of preantennal setae and 4 rows postantennal setae. Clypeal tubercle considerably above antoral margin. Eye moderately pigmented, with a translucent area in the caudal part. Labial palpus five-segmented, extending approximately to the apex of the forecoxa. Antenna (Fig. 1, E) with scape bearing about 6 short marginal setae; setae of pedicel short, not extending beyond the base of the club.

Pronotal comb with 12 teeth per side; pronotum with 2 rows of setae. Mesonotum with 4 poorly-defined rows of fine setae and a caudal (marginal) row of alternating heavy and fine setae; 1 strong pseudoseta per side. Mesepisternum with about 9 setae, smaller dorsally; mesepimeron with about 12 setae, smaller dorsally. Metanotum with 3 rows of fine setae and a fourth row of heavier setae; lateral metanotal area with a heavy and a fine seta. Metepisternum with a heavy seta near the dorsal margin; metepimeron with 11 setae in three poorly-defined vertical rows, heavier caudally.

Received for publication, December 21, 1953.

PLATE I

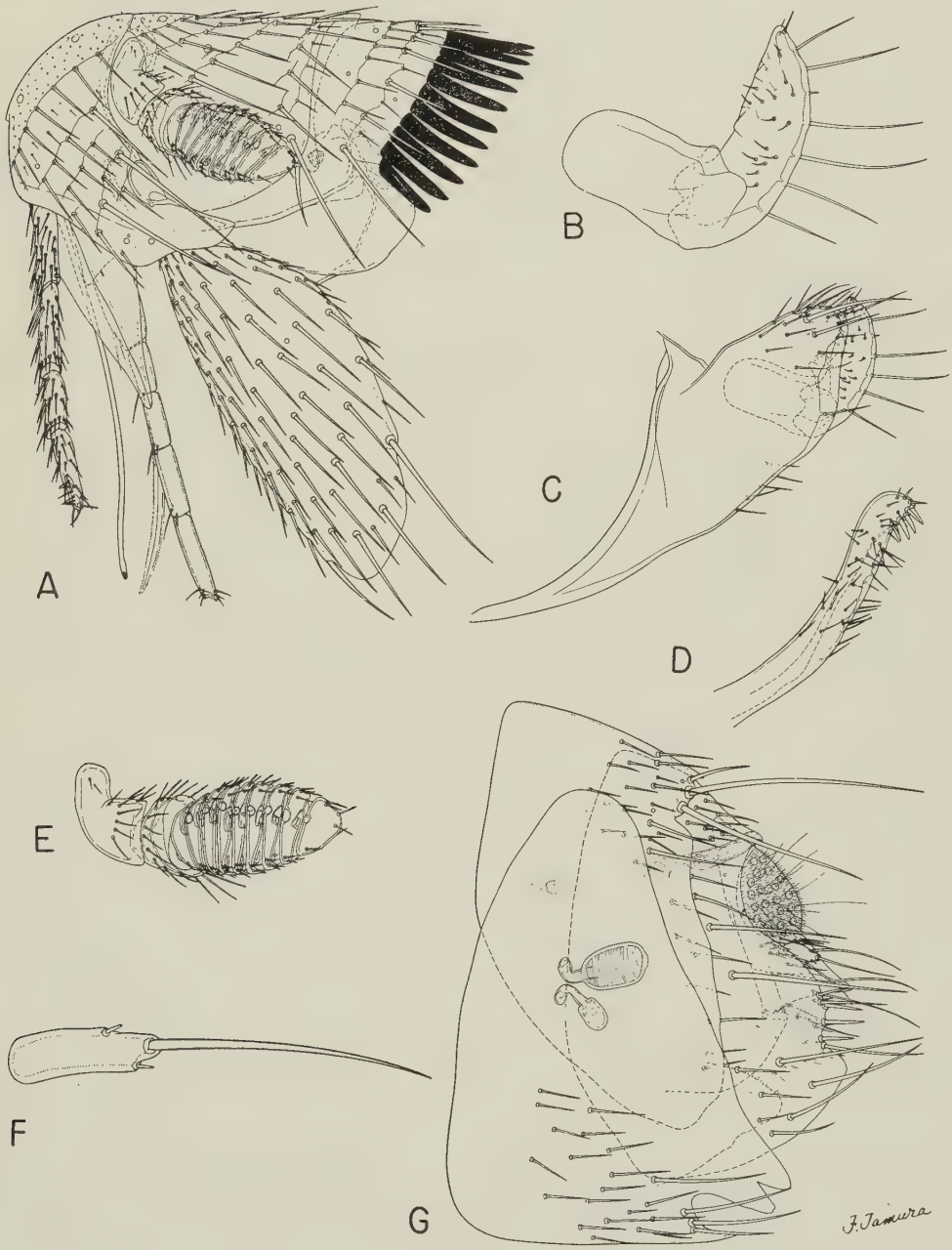


FIG. 1. *Atyphloceras shogakii*, new species: A, head, prothorax, and coxa I of male; B, moveable finger; C, clasper and moveable finger; D, sternite IX of male; E, antenna of male; F, anal stylet of female; G, terminal segments and seminal receptacles of female.

Tergum I with 3 rows of setae and a single pigmented tooth near the ventral margin. Tergum II with 2 rows of setae and 2 pigmented teeth near the ventral margin. Terga III-VI each with 2 rows of setae and a single tooth. With 3 large antepygidial bristles. Tergum VII rather expanded, and with an angulate caudal margin.

Forecoxa (Fig. 1, A) as illustrated; mesocoxa with setae anteriorly only; metacoxa with about 30 fine setae mesally on the anterior half, and with a few heavier setae laterally, near the anterior margin. Metatibia with 6 notches on the caudal margin; beginning at the base, the notches contain 2-1-2-2-2-1 setae; near the apex, on the caudal margin, are 1 small and 2 large setae.

Margin of the clasper (Fig. 1, C) entire, convex dorsally, straight and vertical caudally with setae as illustrated. Moveable finger (Fig. 1, B) apparently L-shaped, with small setae scattered laterally and 6 or 7 heavy setae on the convex caudal margin. Sternite VIII evenly rounded, with 4 heavy marginal setae. Sternite IX (Fig. 1, D) rounded and with 3 to 5 short, rounded, pigmented setae ventrally at the apex; smaller setae as illustrated.

Female: In general facies the sexes are similar. Points of difference are noted. Antenna slightly shorter than that of male. Pronotal comb with 12 teeth per side. Mesonotum with 1 or 2 pseudosetae per side.

Abdomen similar to that of male; pigmented teeth only on terga I–V. Sternite VII (Fig. 1, G) with a bisected lobe (more heavily sclerotized) below which is a rather deep sinus. Anal stylet (Fig. 1, F) about 3 times as long as wide, with setae as indicated. Stigma on tergum VIII long, typical for the genus. Seminal receptacles (Fig. 1, C) markedly dissimilar in size. Bursa copulatrix poorly sclerotized.

Types: Holotype male and allotype female from *Apodemus speciosus* (Temminck and Schlegel), Karasawa, Mt. Hodaka (elev. 8200 feet), Nagano Prefecture, 21 August 1953, coll. Tsugio Hirabayashi; deposited in the U. S. National Museum, No. 62233. Paratypes: 1 male and 1 female with the same data as the holotype; 1 male and 1 female from the type locality and type host, 4 August 1953; 1 female from *Apodemus geisha* (Thomas) from type locality, 5 August 1953, coll. Tsugio Hirabayashi; 1 male and 1 female from *A. geisha* (Thomas), 8 August 1953, coll. Tsugio Hirabayashi; 1 female from *A. speciosus* (Temminck and Schlegel), Kamikochi, Nagano Prefecture, 12 July 1952, coll. Mitosi Tokuda and E. W. Jameson, Jr.; 1 female from *A. geisha* (Thomas), Kamikochi, Nagano Prefecture, 12 July 1952, coll. Mitosi Tokuda and E. W. Jameson, Jr.; and 1 male from *A. speciosus* (Temminck and Schlegel), Hakata (Camp Hakata), Fukuoka Prefecture, 26 February 1952, coll. Major A. S. Kidwell and E. W. Jameson, Jr.

Comments: The presence of only 1 or 2 pigmented teeth per side on the abdominal segments is unique in the genus; they are numerous in most species. The female is easily distinguished by the great disparity in size between the 2 seminal receptacles. This condition is known only in *Macropsylla hercules* Rothschild, and and in such species in which a second seminal receptacle is an anomaly; and in this respect *A. shogakii* may represent a rather advanced form of the genus.

The short moveable finger and the short, stout setae on the ninth sternite of the male are also unique in the genus. *A. shogakii* does not appear to be closely related to any other species of *Atyphloceras*.

We take pleasure in naming this species for Dr. Yukio Shogaki who has made many contributions to the study of medical entomology in Japan.

Stivalius aestivalis, new species
Figs. 2, A–D and 3, A–F

Male: Head, prothorax, and coxa (Fig. 2, A) more or less typical for the genus: on the head are 4 rows of prominent preantennal setae, usually 6, 5, 4, and 3 setae to each row (from front to rear); these large setae are somewhat variable in position and there may be an extra seta between rows 3 and 4. Ten to 14 very small setae behind row 1; and 3 or 4 intermediate setae behind row 2. Three rows of postantennal setae: 5, 6, and 6 setae to each row. About 17 to 19 minute setae on dorsal margin of antennal groove. Eye well-pigmented, with a marked ventral sinus. Antennal grooves not confluent dorsally. Labial palpus five-segmented, not extending to apex of forecoxa.

Pronotum (Fig. 2, A) with 2 rows of bristles: an anterior row of about 6 and a posterior row of 6 somewhat larger bristles; ventrally the position of these bristles is variable. Pronotal

PLATE II

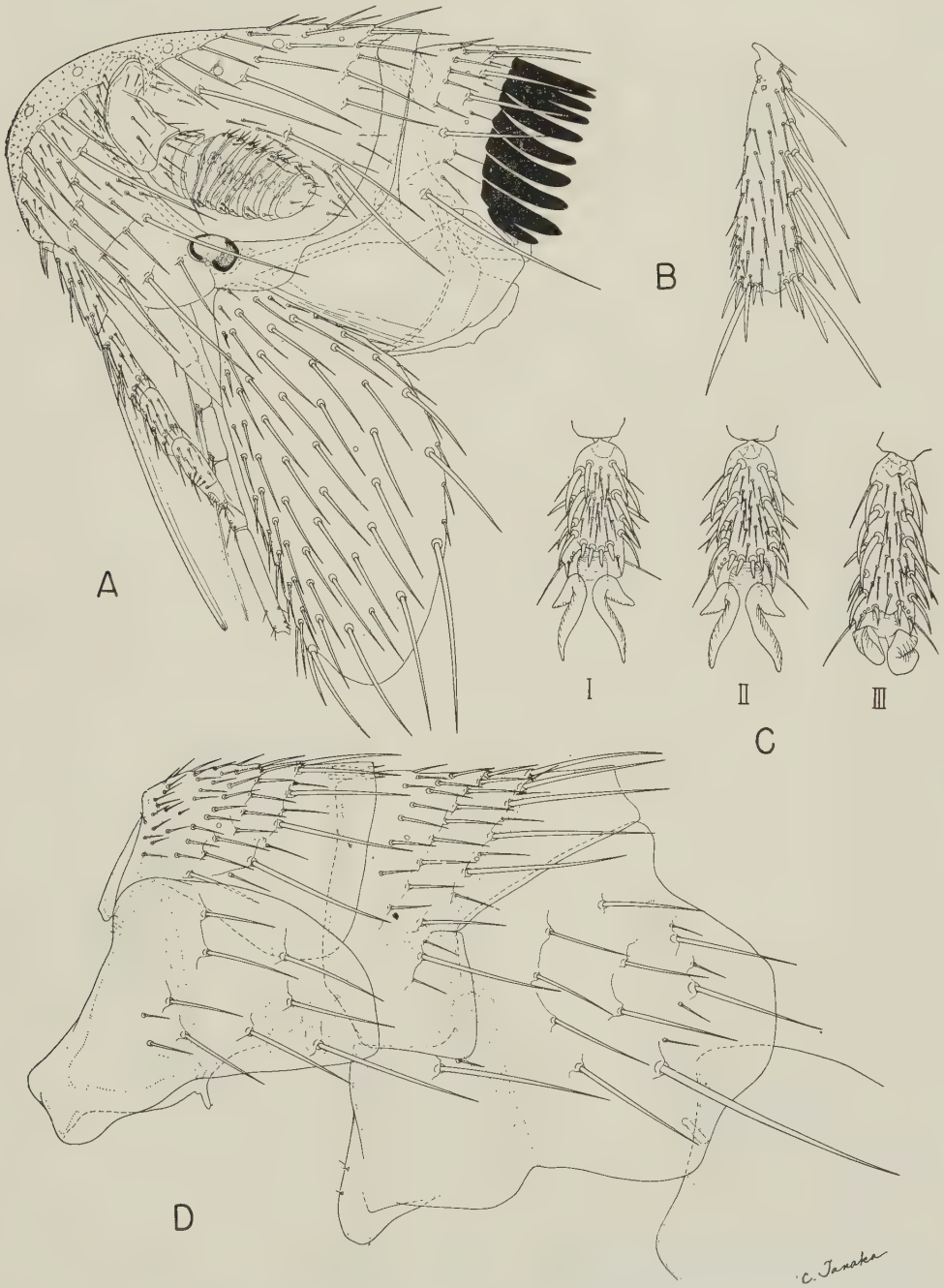


FIG. 2. *Stivalius aestivalis,* new species: A, head, prothorax, and coxa I of male; B, metatibia III of male; C, fifth tarsal segments of male; D, meso- and metathorax of male.

PLATE III

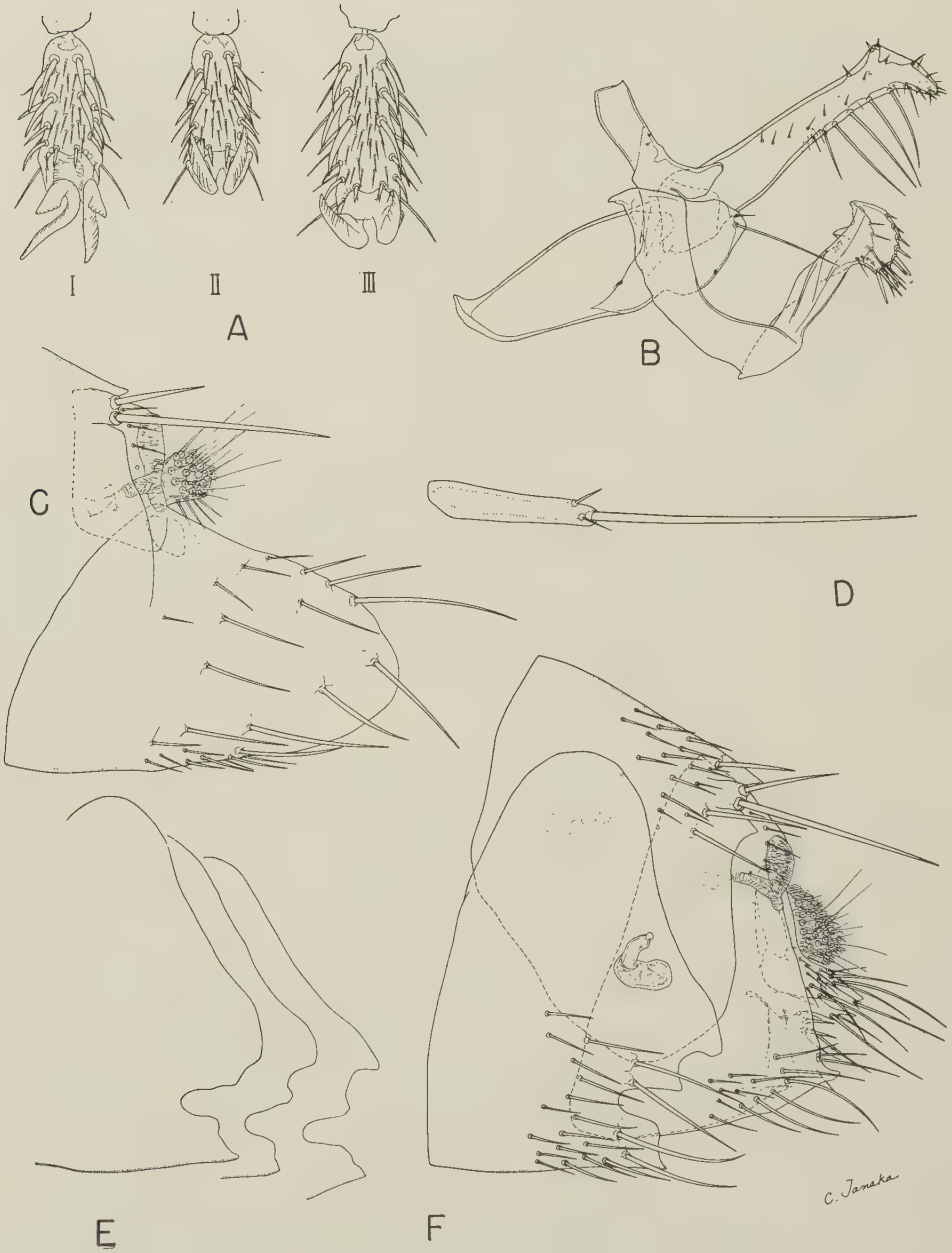


FIG. 3. *Stivalius aestivalis,* new species: A, fifth tarsal segments of female; B, clasper, moveable finger, and sternite IX of male; C. tergites VII and VIII and sternite VIII of male; D, anal stylet of female; E, variations in outline of sternite VII of female; F, terminal segments of female.

comb of about 10 spines or teeth to a side, individual teeth about as long as pronotum. Cervical sclerite hook-shaped, the ends being directed mesad. Mesonotum (Fig. 2, D) with 2 pseudosetae per side, beneath the collar; with 4 rows of setae: 5, 6, 8, and 9 setae per row, the setae increasing in size posteriorly. No apical spinelets or pseudosetae on metanotum. Mesosternum and metasternum with sclerities and setae as figured, similar to *insolli* Traub.

Abdominal terga typically with 2 rows of setae: an interior row of medium-sized setae and a posterior row of alternately large and small setae. Abdominal terga II–V each with 1 apical spinelet per side, placed rather high. With 2 antepygidial bristles, the upper slightly more than half the length of the lower. Spiracle of tergum VIII about the same size of the pygidium.

Fifth tarsal segments as illustrated (Fig. 2, C), with 6 pairs of plantar bristles, placed laterally, but not in straight lines. The stout, ventral, subapical setae of segments V are arranged 4-4-2; those on metatarsal segment V being relatively slender. Metatibia (Fig. 2, B) as illustrated: the number of setae in each dorsal notch variable according to the interpretation of the limits of the notches, but a constant character.

Modified segments as illustrated. Eighth sternum (Fig. 3, C) large, with about 10 setae near the caudal and ventral margins, and about 8 setae near the dorsal margin. Moveable finger (Fig. 3, B) about 8 times as long as wide, straight throughout most of its length, curved ventrally at its apex; with 4 or 5 slender setae along the distal half of the ventral (caudal) margin. Distal arm of sternite IX (Fig. 3, B) with a dilated apical part; laterally on this dilation there is a ridge bearing 5 or 6 slender setae, the dorsalmost being slender and the caudalmost being spiniform. On the anterior margin of this expanded part is a spur-like, sclerotized projection; on the inner surface of this expanded part is a patch of about 6 slender setae.

Female: Essentially like the male except for the terminal segments. Chaetotaxy of head, thorax and abdomen much as in the male. Anal stylet (Fig. 3, D) 4–5 times as long as wide; 2 small subapical setae, and a long apical seta, almost straight, sometimes slightly curved dorsad.

Legs similar to those of male. The stout, ventral, subapical tarsal setae (of segments V) of the male are rather slender in the female (Fig. 3, A) and arranged 2-2-2.

Outline of sternite VII (Figs. 3, E and F) variable: with a marked subventral sinus, and sometimes with a second, shallower sinus, dorsad; sometimes only the ventral sinus is present and the margin dorsad is wavy. Tergum VII (Fig. 3, F) with an acute projection both above and below the antepygidial bristles. Spermatheca slightly concave ventrally (or with the ventral margin irregular) and with a distinct concavity dorsally. Tail at right angles to the body, with a mesal crook.

Types: Holotype male and allotype female from the wood mouse, *Apodemus speciosus* (Temminck and Schlegel) from the wooded hills about 8 miles north of Kyoto City, Kyoto Prefecture; 11 June 1952. Paratypes, all from the type host, with the following data: 4 males and 6 females with the same data as the types; 2 males from the type locality, 1 August 1952; 1 male from Kyoto City, 23 August 1952; 1 male from Camp Fuji (on slope of Mt. Fuji), Shizuoka Prefecture, 28 August 1952; 5 males and 5 females from Camp Fuji, 29 August 1952; and 2 males and 2 females from Camp Fuji, 30 August 1952; specimens from Camp Fuji collected by Lt. T. C. Sicay.

Holotype, allotype, and 2 pairs of paratypes deposited at the U. S. National Museum, No. 62232. Paratypes deposited at the British Museum (Natural History) at Tring; Institute for Infectious Diseases, University of Tokyo; Entomological Laboratory, Kyushu University, Fukuoka; Department of Entomology, University of Hokkaido, Sapporo; and Canadian National Museum, Ottawa.

Remarks: This species is close to *Stivalius insolli* Traub and *S. klossi* (Jordan and Rothschild). *S. aestivalis* has about 10 pronotal teeth per side as in most members of the genus, but *insolli* has about 14 teeth per side. In *aestivalis* the apical crook of the moveable finger is short, relative to the thickness of the finger itself. The distal arm of sternite IX is rather abruptly dilated; the setae on the caudal margin of the dilation are thicker at the base in *aestivalis* and thicker apically in *insolli*. In *klossi* the apex is gradually rounded, dilated ventrally. The outline of sternite VII of the female is quite variable in *aestivalis;* 2 sinuses or a subventral sinus and a wavy margin dorsad, as illustrated. The dorsal concavity of the body of the spermatheca is distinct for *aestivalis*.

The name refers to the seasonal distribution of this species—*Stivalius aestivalius* has been collected only from June until August; this species was not found from

PLATE IV

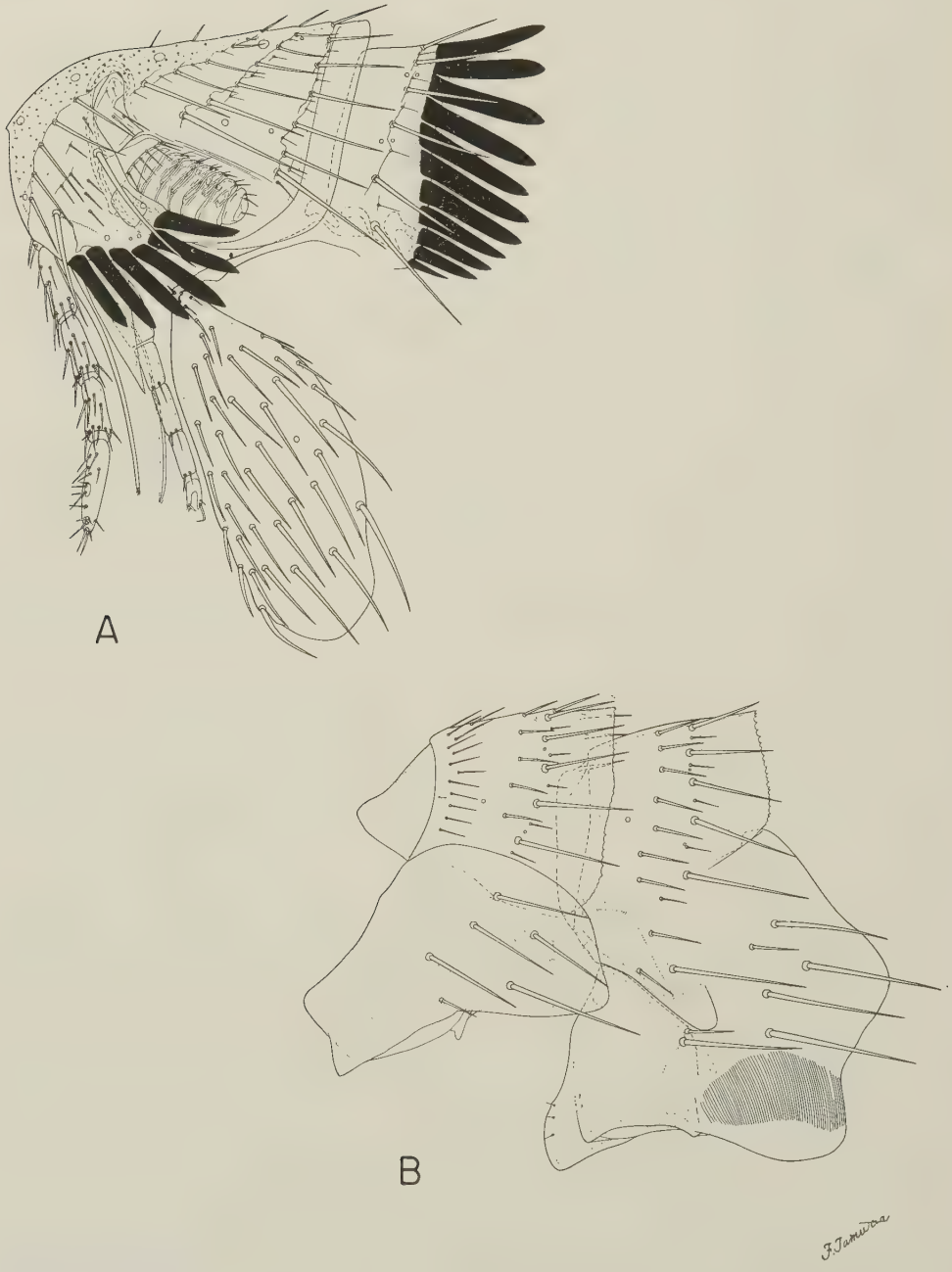


FIG. 4. *Rhadinopsylla (Rectofrontia) attenuata,* new species: A, head, prothorax, and coxa I of male; B, meso- and metathorax of male.

PLATE V

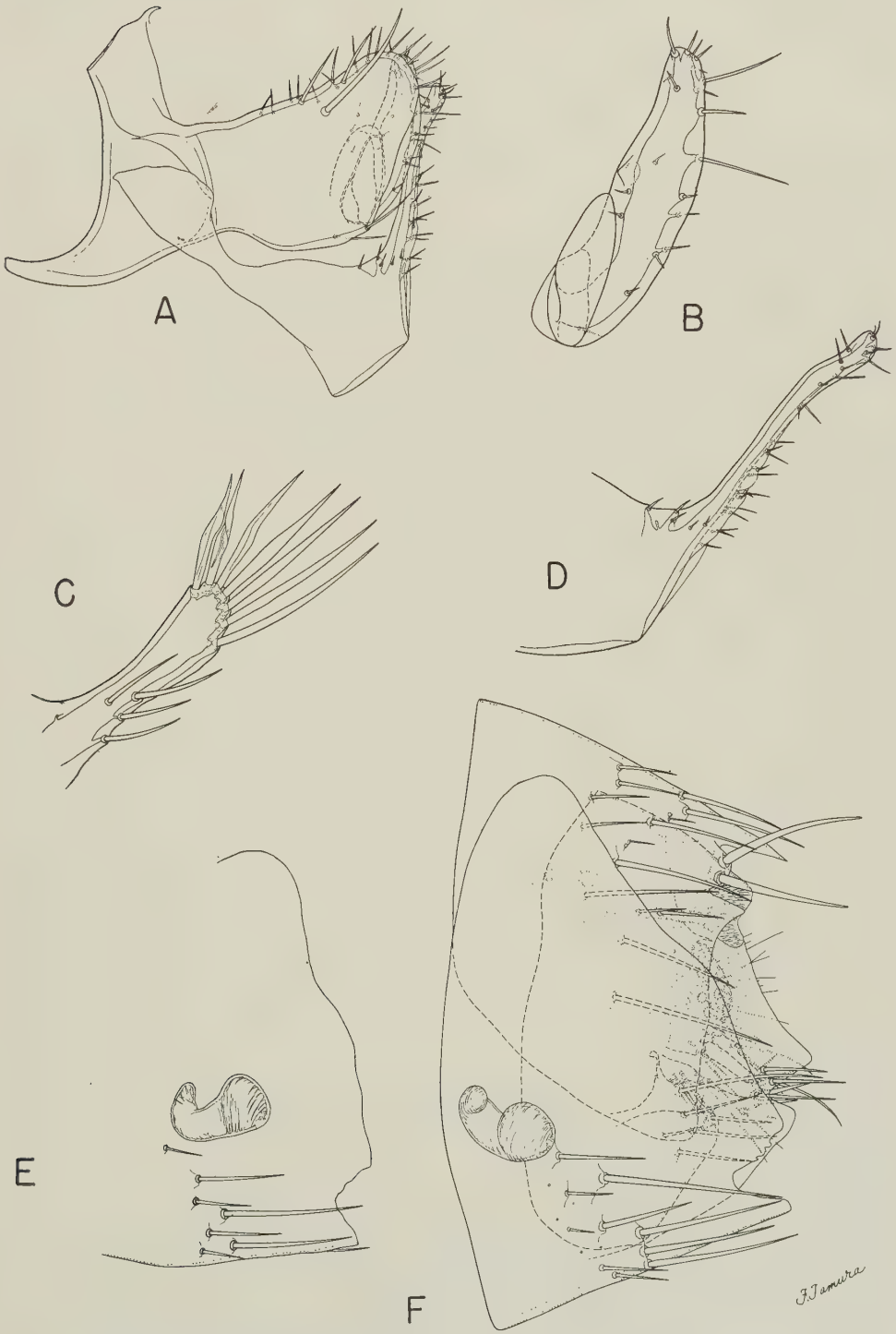


FIG. 5. *Rhadinopsylla (Rectofrontia) attenuata,* new species: A, clasper, moveable finger, and sternite IX of male; B, moveable finger; C, sternite VIII of male; D, sternite IX of male; E, sternite VII and seminal receptacle of female; F, terminal abdominal segments of female.

September through March on the type host many specimens of which were examined for external parasites.

Rhadinopsylla (*Rectofrontia*) *attenuata*, new species
Fig. 4, A–B and 5, A–F

An unnamed species of *Rhadinopsylla* is one of the less frequently found fleas on Japanese mice. The specimens are from *Clethrionomys smithii* (Thomas) and *Apodemus speciosus* (Temminck and Schlegel); all of the hosts came from the high hills or mountains.

Male: Head (Fig. 4, A) with an acute frontal angle and 6 genal teeth. There is a preantennal row of 5 moderate setae, 2 considerably longer setae posteriorly, and 6 or 7 very short setae between. Three rows of post-antennal setae: first row of 5 long setae alternating with 5 minute setae, the long setae extending beyond the bases of the setae in the second row. Eye absent. Pedicel of antenna without long setae; inner face of the club of antenna with moderately long setae on the distal half.

Pronotum (Fig. 4, A) with 11–12 heavy, pigmented spines per side. Mesonotum (Fig. 4, B) with 3 rows of setae and 3 pseudosetae per side, under the collar. Metanotum with 2 rows of setae and no pseudosetae. The sclerotic ridges and divisions of the thorax, as illustrated, are specifically constant and distinct from allied species.

Coxa III with a mesal patch of about 12–15 small, stout setae. Posterior margin of hind tibia with 6 pairs of long heavy setae, 8 smaller lateral setae and a pair of long, heavy setae anteriorly at the apex. Fifth tarsal segment of each leg with 4 pairs of lateral plantar bristles.

Each abdominal tergite with 2 rows of bristles and 2–4 pigmented, marginal teeth (4-4-4-4-3-3-2 in the holotype). Abdominal sternites with 3–5 setae ventrally.

Fixed process of clasper (Fig. 5, A) oblong, with a few delicate setae dorsally and caudally and with 1 larger lateral seta. Moveable finger extending almost to the dorsal margin of the fixed process; oblong, slightly convex posteriorly and with setae as illustrated (Fig. 5, B). Eighth sternite (Fig. 5, C) slightly expanded and with 6 or 7 flattened and expanded setae. Distal arm of the ninth sternite (Fig. 5, D) long and slender, and with small scattered setae on the caudal margin.

Female: Female similar to the male in most features. Six genal teeth as in the male. Two antepygidial bristles per side. Seventh sternite (Fig. 5, E and F) with a moderately shallow ventral sinus; for a short distance dorsad from this sinus the margin is ragged and appears broken. The base of the anal stylet is about 4 times as long as wide; there are two small subapical bristles ventrally, and a long terminal bristle. Body of seminal receptacle somewhat bulbous, the neck small and tapering.

Types: Holotype male from *Clethrionomys smithii* (Thomas), Honshu, Kyoto Prefecture, Ohara, 7 March 1952, coll. E. W. Jameson, Jr. Allotype female from the same host and locality, 3 April 1952. Paratypes as follows: 2 females with data as holotype, 7 and 8 March 1952; 1 female from *C. smithii,* Hanase, Kyoto Prefecture, 11 June 1952, coll. E. W. Jameson, Jr.; 1 female from *C. smithii,* Mt. Hiei, Shiga Prefecture, 27 March 1952, coll. Dr. Mitosi Tokuda; 1 male from *Apodemus speciosus* (Temminck and Schlegel), Mt. Fuji, Shizuoka Prefecture, 18 December 1952, coll. Lt. T. C. Sicay; and 1 female from *A. speciosus* Hata-Mura, Nagano Prefecture, 27 November 1952, coll. Tsugio Hirabayashi. Holotype, allotype and one paratype deposited in the U. S. National Museum, No. 62231. Paratypes deposited in the British Museum (at Tring) and in the collections of the authors.

Remarks: The diagnostic features of *Rhadinopsylla attenuata* are found in the head and thorax and in the genitalia. The shape of the head and thoracic segments cannot be clearly put into words, but the characters are shown in the figures. The inflated setae of sternite VIII of the male are distinct for the genus. Sternite IX of the male is similar to that of *R. tenella* Jordan, 1929, but is clearly different.

This paper is based in part upon studies which were conducted under contract no. DA-49-007-MD-242, between the Regents of the University of California and the Department of the Army. The work was initiated while the senior author was associated with the 406th Medical General Laboratory and Far East Medical Research Unit in Japan during the period January–October 1952. Logistical support

for the preparation of the illustrations was supplied by the U.S. Army Hospital, 8164 Army Unit, APO 9, by special arrangement with the above-mentioned organizations.

We wish to thank Dr. Karl Jordan and Mr. F.G.A.M. Smit for generous help during the course of our work. It is a pleasure to thank Miss Fujiko Tamura and Miss Chieko Tanaka for the excellence of the accompanying illustrations.

Literature Cited

Hopkins, G. H. E. 1952 Notes on synonymy in Siphonaptera. J. Wash. Acad. Sci., **42**: 363-365.

A STUDY OF SEVERAL FACTORS INFLUENCING HATCHING OF *SCHISTOSOMA JAPONICUM* EGGS

Saburo Sugiura, Takashi Sasaki, Yukio Hosaka and Ryozo Ono

Department of Schistosomiasis
Yamanashi ken Medical Research Laboratory
Kofu City, Yamanashi ken, Honshu, Japan

INTRODUCTION

Research workers have met with unexplainable and inconsistent hatching failures on the part of eggs of *Schistosoma japonicum,* even when the feces were obtained from the same individual animal and treated according to known optimum conditions. Although these inconsistencies are undoubtedly due in part to fluctuations in egg numbers, unrecognized factors probably still exist. Only a few investigators have dealt with this problem, yet it is important that the student of schistosomiasis be aware of all factors in this regard—and the diagnostician as well, since hatching can serve as a diagnostic aid.

Currently, a number of factors have been considered, including light, temperature and pH of the water, which were previously tested by Miyagawa (1916), Faust and Meleney (1924), Magath and Mathieson (1946), Stunkard (1946), and Ingalls, Hunter, McMullen and Bauman (1949). In addition, attempts were made to reach some conclusion concerning the influence which might be exerted by the design of the hatching chamber, the effect of centrifugation as a mechanical stimulus to hatching, the influence of fecal decomposition, hatchability of eggs from different hosts, and the influence of feces from varied uninfected hosts on eggs from a single animal.

METHODS

Unless otherwise designated, the eggs used were taken from bloody mucoid stools of an experimentally infected goat. The eggs were carefully washed, separated from the stool medium by the use of micropipettes and transferred to the experimental receptacles, either flask, test tube or watch glass. Unless the experiment was planned otherwise, the receptacles were maintained under optimum conditions of light, temperature and pH. Accurate counts of the eggs were made and their viability was assured by noting motility of miracidia within the shell. During each experiment, miracidia were recovered at intervals not exceeding their life-span, and accurately counted. Where necessary, contents of the incubation chamber were

Received for publication, November 10, 1953.

formalized at the end of the experiment and centrifuged, and the sediment examined for overlooked miracidia and unhatched eggs.

EXPERIMENTAL APPLICATION OF FACTORS AND RESULTS OBTAINED

Design of Hatching Chamber.

As noted by Ingalls *et al.* (1949) the amber colored flask with a transparent side arm, used by McMullen and Beaver (1945) for hatching and concentrating the highly phototropic miracidia of bird schistosomes, was ineffective for the less light-sensitive miracidia of *S. japonicum;* not more than 10% of the miracidia were recovered from the side arm. The hatching chamber and procedure developed by Stunkard (1946) and Ingalls *et al.* (1949) involving a flask supporting a vertical glass tube, adapted by means of a conically-bored rubber stopper, effectively concentrated the negatively geotropic miracidia of *S. japonicum.* Florence and Erlenmeyer flasks were equally effective as the hatching chamber, essentially all the miracidia being recovered from 100 eggs introduced into each type.

The Effect of Temperature on the Hatching Rate.

One hundred eggs were placed in each of a series of 30 ml. test tubes and subjected to temperatures ranging from 2° C. to 37° C. (See Table 1). Eggs hatched

TABLE 1.—*Effect of temperature*

Temperature degrees C.	No. eggs	Miracidia recovered	Eggs remaining
35–37	100	7	93
30	100	87	13
25	100	88	11
15	100	80	17
10	100	69	27
6–8	100	23	74
2–4	100	3	97

at these extremes, but at a very low rate, the optimum range being 15° to 30° C. Considerable hatching occurred at 10° C., but decreased sharply below this point. Previously Miyagawa (1916), Magath and Mathieson (1946), and Ingalls *et al.* (1949) noted 25° to 30° C. as being either favorable or optimal. Faust and Meleney (1924) established an optimum range of 8° to 37° C. Current results more nearly favor those of latter investigators. It would seem then that temperature is not a critical factor under natural conditions.

The Effect of Light on Hatching of Eggs Recovered from Six Different Hosts.

Viable eggs were obtained from a naturally infected cow, dog and man, and from an experimentally infected rabbit, goat and cat. For the latter three, eggs were recovered 70 days after exposure. The washed eggs were introduced into small dishes containing 2 ml. of water which was changed every 24 hours for a period of 5 days. One dish was placed in the light on the window sill while the other dish was placed inside a light-tight chamber. The data (Table 2) show that hatching is somewhat suppressed in darkness. Of those eggs maintained in light, 76% hatched in contrast to 48% of those in darkness, a difference of 28%, which constitutes about a 60% increase, presumably due to light. It should be noted that these findings are not in accord with those of Ingalls *et al.* (1949), who reported

TABLE 2.—*Effect of light*

Incubation period of 120 hours			Total miracidia—120 hours	
Egg source	Light or darkness	No. eggs	No.	%
Rabbit	Light	153	116	76
	Darkness	42	18	43
Goat	Light	102	80	78
	Darkness	56	26	46
Cow	Light	123	93	76
	Darkness	125	50	40
Cat	Light	211	133	63
	Darkness	210	118	56
Dog	Light	100	76	76
	Darkness	50	24	48
Man	Light	122	121	99
	Darkness	70	27	39
Totals	Light	811	619	76
	Darkness	553	263	48
Ratio	Darkness to Light 1 : 1.6			

that the rate of hatching was not significantly influenced by variations of light intensity.

Determination of the Optimum pH Range.

For this experiment, viable eggs were introduced into a series of test tubes (30 ml.) with a graduation of pH ranging from 2.0 to 8.6, prepared and tested for accuracy by using an S. Z. K. Standard Colorimeter. Below 6.8 the pH was adjusted with sodium citrate, and above 7.0 with calcium carbonate. These buffers were used because they appeared to be less toxic to the miracidia than other chemicals at our disposal.

It may be noted (Table 3) that hatching occurred at a relatively uniform rate over a pH range of 5.0 to 8.0, but was markedly suppressed above and below these levels. The results of this experiment differ from those of Ingalls *et al.* (1949), who reported that a pH below 6.6 was inhibitory and that the best results were obtained at a pH level slightly above 7.6.

The Effect of Centrifugation on Hatching.

Since centrifugation was used in some of these experiments as a method of concentrating and isolating eggs from infected stools, it was deemed advisable to determine whether centrifugation acted in any way to inhibit or increase hatching. Eggs were centrifuged at speeds up to 3000 r.p.m. for 10 minutes, then placed in the light

TABLE 3.—*Optimum pH range*

pH	Number of eggs	Remaining eggs	Miracidia recovered
2.0	100	100*	0
3.0	100	92	5
4.0	100	52	38
5.0	100	33	66
6.0	100	34	66
6.8	100	31	65
7.0	100	46	53
7.2	100	46	53
7.6	100	41	58
8.0	100	40	59
8.6	100	92	3

* Although the miracidium was capable of leaving the egg shell, it was shriveled and unable to escape from the egg shell membrane, and thus was considered unhatched.

on a window sill. Control eggs were handled similarly except for centrifugation. Miracidia were collected and counted every hour for five hours. The results indicate that centrifugation had little or no effect upon hatching.

The Effect of Decaying Fecal Matter Upon Hatching.

It has been assumed by previous workers that fecal decomposition tends to retard hatching. Five ml. of noninfected goat stool was mixed with 300 ml. of water and strained; then, 30 ml. of this strained mixture was placed in a hatching chamber and 300 viable eggs placed in this mixture. During the first 10 days, only a very small number of miracidia hatched. After 10 days, the decomposition was excessive and no further hatching was observed. This tends to confirm the consistent observations of others that great dilution of fecal debris is necessary before hatching occurs freely. The specific suppressing factor, whether products of decomposition, lack of oxygen or otherwise, has not been determined. It is interesting to speculate regarding field conditions under which eggs hatch; little or nothing is known in this regard. In addition to the presence of a stool with eggs in the vicinity of a snail habitat, coincident conditions including rainfall or irrigation must prevail to promote

TABLE 4.—*Hatchability of eggs from different hosts*

Host	Total eggs	Periodicity of hatching (hrs.)							Miracidia	
		1	6	12	24	48	72	120	Total	%
Rabbit	98	7	22	5	12	7	6	11	70	71
Goat	79	41	6	1	4	2	1	1	56	71
Cow	124	23	16	4	16	12	2	0	73	59
Cat	211	15	34	16	27	10	18	8	128	61
Dog	75	15	12	3	19	1	2	0	52	69
Man	96	16	16	5	33	5	1	0	76	79

hatching of eggs and snail exposure. The relative importance of night soil is also unknown.

A Comparison of the Hatching Rates of Eggs Recovered from Different Animal Sources.

An experiment was devised to compare the hatching rates of ova recovered from an experimentally infected goat, rabbit and cat and a naturally infected cow, dog and man. The data, recorded in Table 4, are confirmed by a previous experiment (see Table 2). The most important point of observation is that the eggs from all of these animals apparently hatched freely, and a large majority within 24 hours. Those differences which were noted might readily be explained by the differences in condition of the individual animal, and the duration and severity of the infection. The experimental exposure of the cat was heavy and the infection severe, which may have resulted in a higher percentage of immature eggs.

Comparison of Hatching Rates of Eggs Recovered From a Single Host and Exposed to the Fecal Material of Various Mammals.

Viable, well washed eggs from an experimentally infected goat, were put into an emulsion of feces from various uninfected animals. From each animal, 20 grams of feces were obtained within 24 hours after elimination and diluted with 80 ml. of water. Quantities of 2.5 ml. of this emulsion (strained) were further diluted to 100 ml. and the eggs added. A surface sample (4 ml.) was taken every hour for

15 hours (for results see Table 5). The rate of hatching in the various fecal emulsions decreased in the following sequence: horse, cow, rabbit, goat, sheep, man, dog, cat and mouse. Though naturally infected, eggs are rarely recovered from the horse. The results are relatively consistent in that the rate of hatching is higher in stools of herbivorous than in those of omnivorous and carnivorous animals. The span of hatching occurred over a longer period of time in the herbivore stool. The greater protein intake might be a significant factor in the suppression of hatching in the carnivore stool. In succeeding experiments it was shown that the eggs hatched at an increased rate and over a greater span of time when treated with calcium carbonate.

TABLE 5.—*Hatchability of eggs from a common source when exposed to feces of various mammalian hosts*

Source of feces	Total eggs	Periodicity of hatching (hrs.)							Miracidia	
		2	4	6	8	10	12	15	Total	%
Horse	205	93	37	69	13	2	2	0	176	86
Cow	435	177	66	19	13	7	12	23	317	73
Rabbit	449	104	86	31	11	8	9	0	249	56
Goat	428	99	37	32	26	13	7	1	215	50
Sheep	200	35	1	16	27	0	3	3	85	43
Man	410	72	14	0	0	1	0	0	87	21
Dog	410	74	3	3	6	3	2	3	94	23
Cat	405	41	3	0	0	0	0	6	50	12
Mouse	405	31	14	0	0	0	0	0	45	11

SUMMARY

The hatching chamber consisting of a flask (either Erlenmeyer or Florence) with superimposed glass tube (adapted by a conically bored rubber cork) was highly effective for recovery of *S. japonicum* miracidia *en masse* for experimental needs and for diagnostic purposes. Hatching occurred freely from 10–30° C, with a maximum range of 2–37° C. Although eggs hatched in total darkness, light accounted for a 60% increase. The optimal pH range was 5.0 to 8.0, the extremes being 3.0 to 8.6. Centrifugation had no effect. Fecal decomposition tends to suppress hatching. No significant difference was noted for hatchability of eggs from varied hosts. When eggs from a single animal were mixed with fecal emulsions of varied uninfected hosts, it was noted that feces of carnivores suppressed hatching.

The authors wish to acknowledge their indebtedness to Mr. J. E. Williams, Miss Y. Otori and Dr. L. S. Ritchie of the 406th Medical General Laboratory, and to Dr. D. B. McMullen, Army Medical Service Graduate School, who assisted in translating and editing the manuscript.

REFERENCES

FAUST, E. C. AND MELENEY, H. E. 1924 Studies on schistosomiasis japonica, etc. Am. J. Hyg. Monogr. Ser. **3**, 399 pp.

INGALLS, J. W., JR., HUNTER, G. W. III, MCMULLEN, D. B. AND BAUMAN, P. M. 1949 The molluscan intermediate host and schistosomiasis japonica 1. Observations on the conditions governing the hatching of the eggs of *Schistosoma japonicum*. J. Parasit. **35**: 147–151.

MAGATH, T. B. AND MATHIESON, D. R. 1946 Factors affecting the hatching of ova of *Schistosoma japonicum*. J. Parasit. **32**: 64–68.

MCMULLEN, D. B. AND BEAVER, P. C. 1945 Studies on schistosome dermatitis. IX. The life cycles of three dermatitis-producing schistosomes from birds and a discussion of the subfamily Bilharziellinae (Trematoda: Schistosomatidae). Am. J. Hyg., **42**: 128–154.

Miyagawa, Y. 1916 Ueber die Veränderungen der Eier des *Schistosomum japonicum* unter den Einwirkungen verschiedener physikalischen und chemischen Agenzien und über einige prophylaktische Massregeln der Schistosomiasis. Mitt. Med. Fak. Univ. Tokyo **15:** 453–475.

Stunkard, H. W. 1946 Possible snail hosts of human schistosomes in the United States. J. Parasit. **32:** 539–552.

PARASITES OF THE GOLDEYE, *HIODON ALOSOIDES* (RAF.), IN LAKE TEXOMA

J. Teague Self

Department of Zoology and Lake Texoma Biological Station, University of Oklahoma

Beginning with the summer of 1949, continuous collections of parasites have been made from the goldeye, *Hiodon alosoides* (Raf.) as a part of studies being conducted on the parasites of Lake Texoma fishes. Intensive collections were made through each of 3 succeeding summers as well as monthly collections during other seasons.

A majority of the more than 200 hosts examined were infected in the intestine with many specimens of an undescribed pseudophyllidean cestode belonging to the genus *Bothriocephalus* Rudolphi and a papillose fluke, *Crepidostomum illinoiense* Hopkins. The only other parasites we recovered from these fish were 4 immature nematodes belonging to the family Camallanidae. It is assumed that the goldeye is an abnormal host for these nematodes since only 4 immature specimens were recovered.

Bothriocephalus texomensis n. sp.
Figs. 1, 2, 3.

Specific diagnosis: Maximum length 220 mm. Maximum width 1.50 mm. Scolex of largest specimen 0.85 mm. long. Bothria deeply grooved, extending length of scolex. Terminal disc on scolex unlobed. Width of neck at junction with scolex 0.26 mm.; its length to first genital rudiment 54 mm. Width of strobila at posterior end of neck 0.80 mm. Length of youngest proglottids 0.70 mm. Mature proglottids 1.22 mm. in width by 1.00 mm. in length.

Ovary bilobed, each lobe 0.20 mm. by 0.10 mm. Oötype almost spherical, about 0.10 mm. in diameter. Vitellaria composed of 400 to 450 follicles, each follicle about 39 microns in diameter. Testes, 79 microns in greatest diameter, numbering 50 to 60 in two lateral fields, each field lying on both sides of lateral nerve cord; greater number median to nerve cord. Cirrus sac large, thin-walled with maximum length of 0.25 mm. Cirrus never observed to protrude. Vagina opening to outside close behind cirrus opening. Uterine duct confined to one side, alternating irregularly. Uterine sac spherical, its maximum diameter one-third that of proglottid. Uterine pore near anterior margin of proglottid. Genital opening median, just anterior to mid-length of proglottid. Longitudinal muscular fibers present, but not in bundles. Two main lateral nerve cords and 2 principal lateral excretory canals present. Secondary excretory canals present, but number not consistent from one segment to the other. Calcareous bodies absent. Each original proglottid subdividing into 8 subsegments.

Host: Hiodon alosoides (Raf.).

Organ: Intestine.

Locality: Lake Texoma, Oklahoma.

Types: Type specimen, U. S. Nat. Mus. Helm. Coll. No. 49412, paratypes in Parasitology Coll., Univ. of Oklahoma Museum.

DISCUSSION

This worm is most closely related to *Bothriocephalus cuspidatus* Cooper, particularly as the latter is figured by Van Cleave and Meuller (1934). However, it differs from *B. cuspidatus* in several respects. It has a dominant terminal disc on the scolex which is not bilobed. The cirrus was not observed to protrude. The

ovarian isthmus is thin and almost indistinguishable. The number of vitelline follicles is 400 to 450 instead of 800 to 1000. They average 38.8 instead of 90–100 microns in diameter. The vitellaria occupy 2 distinct lateral fields and are never united either dorsally or ventrally. The uterus is seldom expanded into a sac and there is no horizontal elongation. There are always 8 instead of 32 subsegments as described by Cooper (1919).

It is recognized that there is much intra-species variation among the bothriocephalids and that one must be cautious about describing new species in the group. Only after studying hundreds of fixed and living specimens am I convinced that the parasite under consideration represents a new species. The scolex of *B. texomensis* is roughly rectangular in shape, being somewhat pointed posteriorly, and is never "arrow shaped" as described by Cooper (1917) for *B. cuspidatus*. The first segments are quite distinct from the scolex posteriorly but are never more than slightly wedge shaped. Subsegmentation occurs regularly, each original segment producing 8 subsegments as opposed to the 32 subsegments produced in each original segment in *B. cuspidatus*.

When this worm is compared to the closely related subspecies of *B. cuspidatus* established by Wardle (1932) one finds no consistent relationship between it and any one of the three subspecies. It differs from *B. c. hiodontus,* the subspecies previously reported from *Amphidon* (= *Hiodon*), in that in the latter, the apical disc is inconspicuous, the bothria shallow and the scolex is relatively short and pointed anteriorly. The scolex is somewhat like that of *B. c. luciopercae* in shape, but the two worms differ in the characters mentioned above.

Crepidostomum illinoiense Hopkins
Figs. 4 and 5

The author has enjoyed the advantage of having large numbers of both living and stained specimens of *C. illinoiense* for study, and the extreme variations noted for their general morphological features have been forcefully apparent. Because of these variations it has been difficult to arrive at a specific determination. However, these worms are identified as *C. illinoiense* on the basis of the notched condition of the median dorsal papillae and because they conform to the description of that species given by Hopkins (1934) for such important characters as the genital organs. It is easily understood, however, how there could have arisen the confusion in the taxonomy of this species mentioned by Hopkins. In studying dozens of living specimens, as well as numerous stained individuals fixed under conditions of relaxation and contraction, I have observed extreme variation in general shape and appearance. An active specimen, when dropped into a fixative or fixed under a cover glass, will contract to such a degree as to have little superficial resemblance to one removed from the host and allowed to become completely inactive before fixation. As Hopkins indicated, the variations observed by other workers probably were due to varying conditions of their specimens when fixed.

Dr. Hopkins was kind enough to loan me his entire collection of these flukes, including the cotypes used in his studies. Examination of these specimens shows that they are in a contracted condition and resemble my specimens fixed before relaxation. A careful study of Hopkins' specimens, along with my own, clearly establishes my specimens as *C. illinoiense.* Since his description was based on strongly

contracted specimens only, an emendation is given here to attain a more correct diagnosis of the species:

1. Maximum length 5.5 mm. instead of 1.2 mm.
2. Maximum width 0.48 mm. or 0.088 of the length in relaxed specimens as compared to 0.25 of length in contracted specimens.
3. Esophagus 5 times instead of 3 times the length of the pharynx.
4. Pharynx one-fourth instead of one-half the diameter of the oral sucker.
5. A distinct neck behind the oral sucker extends to the crural fork (Fig. 4). Width of neck at level of pharynx 0.19 mm. or 0.4 maximum width of body.

Relaxed specimens have been deposited in the U. S. N. M. Helm. Coll. No. 49413.

The neck region is distinctly narrower than the oral sucker and is a distinctive feature of this species. It is not lost even in highly contracted specimens. When the fluke becomes contracted, the region of the oral sucker often becomes folded under the neck to give the appearance of a much shortened anterior end (Fig. 5). As Hopkins has stated, under this condition the esophagus becomes folded and appears to be much shorter that its actual length.

All other characters are as Hopkins described them and need not be repeated here.

This record of *C. illinoiense* in *Hiodon alosoides* constitutes a new host record for the parasite, although it was reported from *Hiodon tergisus* by Pearse (1924). It has not previously been reported in Oklahoma.

SUMMARY

The goldeye (*Hiodon alosoides*) in Lake Texoma, harbors large numbers of the cestode *Bothriocephalus texomensis* n. sp. and the trematode *Crepidostomum illinoiense*.

The newly described cestode is most closely related to *B. cuspidatus* Cooper, differing from the latter in the nature of the scolex, the number, size, and arrangement of the vitellaria, the failure of the cirrus to protrude, and the smaller number of subsegments formed.

The specific diagnosis of *C. illinoiense* is expanded to describe the variations between relaxed and contracted conditions.

Literature Cited

Cooper, A. R. 1917 A morphological study of bothriocephalid cestodes from fishes. J. Parasit. **4**: 33–39.

———1919 North American pseudophyllidean cestodes from fishes. Ill. Biol. Monog. **4**(4): 288–541 (243 pp.)

Hopkins, S. H. 1934 The papillose Allocreadiidae. Ill. Biol. Monog. **13**: (2) 45–124 (80 pp.).

Pearse, A. S. 1924 Observations on parasitic worms from Wisconsin fishes. Tr. Wisconsin Acad. Sc., Arts and Lett. **21**: 147–160.

Van Cleave, H. J. and Mueller, J. F. 1934 Parasites of the Oneida Lake fishes. Part III. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Annals **3**: 161–334.

Wardle, R. A. 1932 The cestoda of Canadian fishes. II. The Hudson Bay drainage system. Contrib. Canad. Biol. and Fish. 7 (art. 30) Ser. A., Gen. No. 27: [377] 403.

Explanation of Figures

Fig. 1. Mature proglottid of *B. texomensis*. t. testes, v. vitelline follicles, and n. nerve cord.

Fig. 2. Cross section of mature proglottid of *B. texomensis*.

Fig. 3. Scolex of *B. texomensis*.

Fig. 4. *Crepidostomum illinoience*, anterior portion in relaxed condition showing distinct neck.

Fig. 5. *C. illinoiense*, anterior portion in contracted condition.

PLATE I

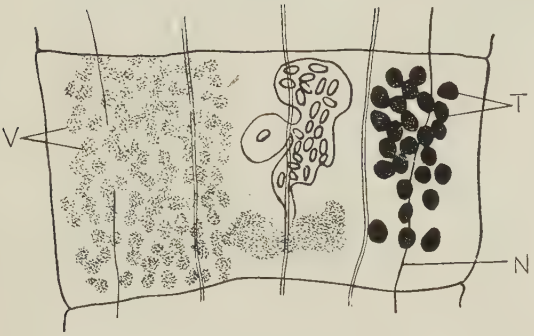


Fig. 1

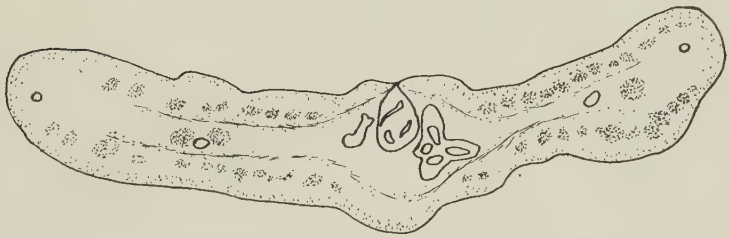


Fig. 2

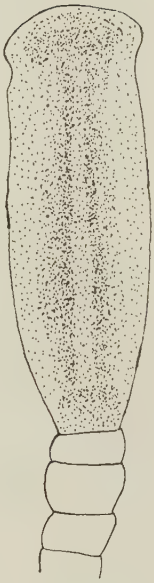


Fig. 3

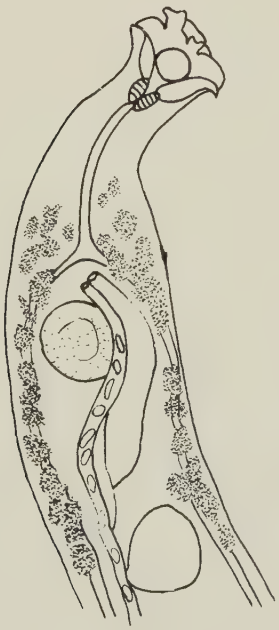


Fig. 4

Fig. 5

THE PRESENCE OF *ENDAMOEBA HISTOLYTICA* IN THE LIVER OF GUINEA PIGS WITH EXPERIMENTAL INTESTINAL AMEBIASIS

CHARLES W. REES, D. JANE TAYLOR, AND LUCY V. REARDON

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute[1], Bethesda, Maryland

Within the last 4 years, the guinea pig has been found to be a satisfactory experimental host for *Endamoeba histolytica* (Carrera and Faust, 1949; Taylor, Greenberg, Highman, and Coatney, 1950). This finding has greatly facilitated studies on the pathogenicity and chemotherapy of amebiasis (Taylor, Greenberg, and Josephson, 1952; Taylor and Greenberg, 1952). The lesions produced by *E. histolytica* in the cecum and colon of the guinea pig are often very severe. However, incidental examination of the liver has shown no macroscopic lesions, and microscopic study of smears of fresh liver tissue has failed to reveal the presence of amebas even in guinea pigs that were killed in the agonal stages of amebic dysentery. Because of the great importance of the question of whether *E. histolytica* may be frequently transported to the liver from intestinal lesions, it was decided to obtain more precise information on this point. Slices of liver, approximately 1 by 1 by 0.5 cm. in size, from intracecally infected animals were inoculated into culture medium in order to detect the parasite. The cultures were seeded with organism *t*, incubated at 37° C. and examined for as much as 144 hours for growth of amebas. In addition, through the courtesy of Dr. Benjamin Highman (Laboratory of Pathology, National Institute of Arthritis and Metabolic Diseases), sections of the liver from some of the animals were examined histopathologically for evidence of damage. This preliminary report presents the data thus far obtained.

The data summarized in Table 1 show that the livers of 15 of the 20 guinea pigs of this experimental series were found infected with *Endamoeba histolytica*.[2] Seventy cultures, or 18.6 percent of 377 tubes inoculated, were found positive for amebas. In many cases the amebas were found only after incubation for 96 or 144 hours, and in some cases only in transfers from the original cultures. Cultures were reported negative only after examination several times between 72 and 144 hours. The time elapsed between the experimental inoculation and post-mortem examination of the guinea pigs is shown in Table 1. The earliest examination was performed 7 days after injection; in this case the liver was found infected, by culture, and the intestinal lesions were graded as most severe. As yet, however, the data are too meager to indicate a relationship between the severity of the intestinal lesions and the occurrence of the parasite in the liver. In a series of cases not tabulated here, there was no parasitism of the liver when the intestinal infection was detectable only by finding parasites within the lumen without demonstrable lesions in the wall. Data from the table show that the left lobe of the liver was found infected, in some cases even when the right lobe was not invaded. The proportion

Received for publication, February 17, 1954.

[1] Laboratory of Tropical Diseases.

[2] All the infections were with NIH strain 200 except for one series in which the F 22 strain was used and the parasite found in the liver.

of left-lobe infections in this series of guinea pigs is higher than that reported for amebic abscesses in the left lobe of human cases.

Nine of the 20 animals were studied histopathologically; 5 of these 9 were found positive by the culture technique. The pathologist's report shows neither amebas nor damage attributable to amebas in any of these guinea pig livers. It is not improbable that these livers were infected with bacteria as well as amebas, but further work is needed for definitive information on this question. Carrera and Faust (1949) reported one incidental finding of infection of the liver with amebas, detected on culture, in a guinea pig with experimental amebic dysentery. The present paper provides confirmation of this finding and shows in addition that under such condi-

TABLE 1—*Results of examination of cultures of whole egg medium for* Endamoeba histolytica, *inoculated with pieces of liver of guinea pigs having amebic dysentery*

Number	Guinea pig		Tubes inoculated		Tubes positive for *E. histolytica*	
	Days since inoculation	Pathogenic index	Right lobe number	Left lobe number	Right lobe	Left lobe
1[1]	18	4[2]	12[3]		1[3]	
2	10	4	6		4	
3	11	4	20		11	
4	19	3	20		7	
5	15	4	10	10	4	0
6	18	3	9	10	3	0
7	19	4	10	10	4	1
8	9	4	10	10	4	0
9	13	4	10	10	1	0
10	18	4	10	10	0	0
11	15	4	10	10	0	0
12	16	4	10	10	3	2
13	18	3	10	10	0	0
14	7	4	10	10	2	3
15	8	4	10	10	0	1
16	9	4	10	10	0	2
17	9	4	10	10	2	5
18	11	4	10	10	0	0
19	13	4	10	10	5	5
20	14	4	10	10	0	0
Totals 20			159	58 160	28	23 19

[1] Died during night, autopsied several hours after death.
[2] Calculated on an arbitrary scale of 1 to 4; 3 indicates gross lesions in the cecum with demonstrable amebas in the fresh smear; 4 indicates that, in addition, there were gross lesions in the colon.
[3] No distinction made between right and left lobes.

tions passage of amebas from lesions in the intestinal tract to the liver may be of frequent occurrence. The data suggest the possibility that human intestinal amebiasis may be frequently accompanied by infection of the liver, and this information may have bearing on the elucidation of the etiology of cases of hepatitis currently considered as of unknown origin.

REFERENCES

CARRERA, G. M. AND FAUST, E. C. 1949 Susceptibility of the guinea pig to *Endamoeba histolytica* of human origin. Am. J. Trop. Med. **29:** 647–667.

TAYLOR, D. J., GREENBERG, J., HIGHMAN, B., AND COATNEY, G. R. 1950 Experimental infection of guinea pigs with *Endamoeba histolytica*. Am. J. Trop. Med. **30:** 817–827.

———, ———, AND JOSEPHSON, E. S. 1952 The effect of two different diets on experimental amebiasis in the guinea pig and in the rat. Am. J. Trop. Med. & Hyg. **1:** 559–566.

——— AND ——— 1952 Experimental chemotherapy of *Endamoeba histolytica* infections in the guinea pig. Am. J. Hyg. **56:** 58–70.

COMPARATIVE SUSCEPTIBILITY OF COMMON LABORATORY ANIMALS TO EXPERIMENTAL INFECTION WITH *SCHISTOSOMA HAEMATOBIUM*[1]

DONALD V. MOORE AND HENRY E. MELENEY[2]

Brumpt (1928) listed the animals which at that time had been reported by various investigators to have been successfully infected with *Schistosoma haematobium.* This list included the following: various species of monkeys, guinea pigs, white mice, white rats and hedgehogs. Stunkard (1946), in a search for a suitable molluscan host for *S. haematobium* among snails native to the United States, used jerbils and baboons, which had been infected in Egypt, as a source of eggs. Watson, Abdel-Azim and Halawani (1948) used mice as experimental hosts for *S. haematobium* in carrying out chemotherapeutic studies. Standen (1949) reported the successful infection of the golden hamster with *S. haematobium,* but lacked sufficient material to make any observations on the course of the infection in the hamster. In the above reports, no mention is made of the number of cercariae used for exposure or of the resulting worm yield or other pertinent information which is of value in experimental schistosome infections.

In order to maintain experimental *S. haematobium* infections in a laboratory situated outside the endemic area, and to conduct investigations on various biological aspects of this infection, it is of the utmost importance to know which of the common laboratory animals are susceptible to this schistosome, and to determine the expected worm yield and percentage worm recovery following standardized cercarial exposures. The rate of maturation and location of the worms in the host, observations on the gross pathology and the passage of viable eggs in the feces and/or urine of the host constitute essential criteria for the determination of the most favorable laboratory animal to be used for *S. haematobium* infections.

MATERIALS AND METHODS

Cercariae for some of our animal exposures were obtained from *Bulinus truncatus* exposed in Egypt and sent to us by Dr. C. H. Barlow. Other animal exposures were made with cercariae from our own laboratory reared and infected *B. truncatus.* Our stock of snails was started through the courteous cooperation of Dr. C. H. Barlow, and the late Dr. M. Abdel-Azim, both of the Bilharzia Snail Destruction Section of the Ministry of Health, Cairo, and of Dr. Robert E. Kuntz, NAMRU No. 3, Cairo, Egypt. Snails were reared, exposed and maintained according to the method described by Moore, Thillet, Carney and Meleney (1953).

In order to obtain cercariae for animal exposures, shedding snails were placed in a crystallizing dish (50 by 30 mm.) filled about half full with spring water. The dishes were placed under a light on a dark background. We observed that the emer-

Received for publication, February 3, 1954.

[1] From the Department of Preventive Medicine, College of Medicine, New York University. This investigation was supported by research grants (E-27 and G-385) from the National Institutes of Health, Public Health Service.

[2] The authors gratefully acknowledge the technical assistance of Dorothy M. Carney, Carlos J. Thillet, Jr., and Josephine P. Moore.

gence of cercariae from the infected snails takes place between the hours of 11 A. M., and 3 P. M. The counting of cercariae and percutaneous exposure of the animals were done according to the methods described by Moore, Yolles and Meleney (1949) for *S. mansoni*. Worms were recovered by the perfusion technic of Yolles, Moore, DeGiusti, Ripsom and Meleney (1947). In addition to the regular perfusion of liver and mesenteries, a third area, the lower extremities including the pelvic circulation, was perfused after the mesenteric perfusion was completed. This third perfusion was accomplished by tying or clamping the cut end of the portal vein and removing the tie on the vena cava, which is severed at the time of removal of the liver. A second entrance was made in the thoracic aorta for this additional perfusion.

Fecal examinations on all animals were done by the saline sedimentation-hatching technic with a microscopic examination of the sediment prior to hatching.

RESULTS

MICE—A total of 47 albino mice, CFW strain, were each exposed percutaneously to 150 cercariae. The animals were sacrificed at two-week intervals from 6 through 14 weeks after exposure. Table 1 shows the recovery, maturity and distribution of the worms in these mice. The average worm recovery for this group of mice was 5.3 worms per mouse or 3.5% of the cercariae employed for exposure. No mature worms were recovered until 10 weeks when 44% of the worms recovered were mature. At 12 weeks 84% were mature and at 14 weeks all of the worms recovered were mature. This is in agreement with Watson, *et al.* (1949), who state that *S. haematobium* reaches maturity between 80 and 100 days. At 6 and 8 weeks after exposure, a large percentage (78% and 69% respectively) of the worms were recovered from the liver. This is in keeping with the expectation since the schistosomes generally have a tendency to remain in the liver during their maturation. At 10 weeks only 38% of the worms were recovered from the liver, but at 12 and 14 weeks, over 50% of the worms were still in the liver. The residence of more than 50% of the worms in the liver after they have all reached maturity, may be taken as an indication that the mouse is not as favorable a host as one in which the majority of mature worms are found in the mesenteric circulation.

At the time of autopsy, a careful gross inspection was made of the vesicular circulation, prior to perfusions. In one or two instances, after perfusing the lower extremities, one worm or a pair in copula were found in the perfusate, however, since no lesions were seen in the bladder and no worms were observed in the vesicular circulation prior to perfusion, we must assume that the few worms found had been trapped in the loops of intestine after being washed out of the mesenteric circulation during the perfusion. On the basis of our findings, we must conclude that in mice, there is no migration of worms from the mesenteric circulation to the vesicular circulation up to 14 weeks after exposure.

No gross pathology attributable to schistosomiasis was observed until 12 weeks after exposure, when several discrete white spots were observed on the liver in most of the mice examined. At 10 weeks the tissue press preparation of the liver of one animal out of 10 revealed a few non-viable eggs. Twelve weeks after infection all 10 animals showed viable eggs in the liver, 3 also showed eggs in the colon, 1 showed eggs in the liver and lung, and 1 showed eggs in the liver, ileum and colon. Fourteen weeks after infection, the eggs were found only in the liver, ileum and colon with the majority of animals showing eggs in the liver and colon only.

Viable eggs were first found in the feces of infected mice 15 weeks after exposure. This is in accord with the findings of Abdel-Azim and Barlow (1948), who found non-viable eggs in the feces of their infected mice as early as 12 weeks and viable eggs between 14 and 16 weeks after exposure. Egg passage in our mice was quite sporadic and eggs were never very numerous. In one instance, we found viable eggs in the feces of infected mice 62 weeks after infection.

HAMSTERS—A total of 65 hamsters were each exposed percutaneously to 200 cercariae, after clipping the hair from the posterior half of the animal. The animals were sacrificed at two-week intervals from 6 through 20 weeks after exposure with the exception of 7 animals sacrificed at 9 weeks after exposure. Table 2 shows the recovery, maturity and distribution of the worms from these animals. The average worm recovery for this group of hamsters was 33.8 worms per hamster or 16.9% of the cercariae employed for exposure. No mature worms were recovered until 9 weeks after exposure when 4% of the worms were mature. All of the worms were mature at 10 weeks. From 6 through 9 weeks a higher percentage of worms was recovered from the intra-hepatic circulation than from the mesenteric circulation. At 10 and 20 weeks, the worms were equally distributed between liver and mesentery. At 12 weeks a slightly higher percentage of worms were found in the liver, and at 14, 16 and 18 weeks, about twice as many worms were recovered from the mesentery than from the liver. This is in contrast to the findings in mice (Table 1.), and may be interpreted as one indication that hamsters are more favorable hosts than mice for *S. haematobium.*

As with the mice a careful inspection of the vesicular circulation was made at the time of autopsy, and this circulation was perfused after the perfusion of the mesenteric circulation. This area is not included in the results shown in Table 2, because the results were not conclusive and any worms found were included with the mesenteric worms in Table 2. With the exception of the animals autopsied at 6 and 12 weeks, one or two worms were found in the perfusate of pelvic circulation, in 1 to 3 animals examined at each age level. Five pairs of worms were found in this perfusate from 1 animal autopsied 16 weeks after exposure. No eggs were observed in the bladder of this animal, and it is quite possible that these worms had been trapped in the folds of the intestine after the mesenteric perfusion There was no good correlation between the presence of eggs in the bladder, and the finding of worms in the perfusate from the pelvic circulation. The question of the migration of the worms from the mesenteric to the pelvic circulation in hamsters needs further careful consideration.

No gross pathology was observed in the animals until 12 weeks after exposure. At this time, discrete grayish-white areas were observed on the liver and the surface of the liver presented a roughened appearance. In 1 animal 2 amber colored raised areas about 1 mm. in diameter were observed on the lung. These lesions each contained a single egg and are indistinguishable from the lung lesions observed in *S. mansoni* infections, (Moore, *et al.* 1949). Eggs were first observed in the tissue at 10 weeks when they were found in the liver of 5 out of 10 animals examined. One of these 10 animals also showed eggs in the colon. At 12 weeks all 10 animals showed eggs in the liver, 2 had eggs in the colon, and 1 had eggs in the lung. At 14 weeks all 7 animals showed eggs in the liver; 4 had eggs in the colon; 3 had eggs in the ileum and one also had eggs in the lung and bladder. From 16 weeks through

TABLE 1.—*Recovery, maturity and distribution of* S. haematobium *in mice following percutaneous infection*

Duration of infection in weeks	Number cercariae per mouse	Number of mice	Total cercariae used	Number of worms recovered					Maturity of worms percentage		Distribution of worms percentage	
				Total	Mature		Immature					
					liver	mes.	liver	mes.	mature	immature	liver	mes.
6	150	8	1200	51			40	11		100	78	22
8	150	10	1500	49			34	15		100	69	31
10	150	10	1500	45	1	19	16	9	44	56	38	62
12	150	10	1500	55	27	19	9		84	16	65	35
14	150	9	1350	48	27	21			100		56	44
Total		47	7050	248								
Average worms per mouse—5.3												
Percentage worm recovery—3.5%												

20 weeks, all animals examined showed eggs in the liver and colon, all but 4 of the animals also showed eggs in the ileum. Eggs were also found in the lungs of a few of the animals, and in one instance eggs were seen in the jejunum. As indicated above, eggs were not found in the bladder until 14 weeks after exposure, indicating that the migration of the worms from mesenteric to pelvic circulation may take place between 12 and 14 weeks. The fact that from 14 through 20 weeks only 3 animals out of a total of 26 examined showed eggs in the bladder, indicates that the migration of the worms into vesicular circulation is only sporadic, at least up to 20 weeks. In a previous study on the histopathology of experimental schistosome infections (Meleney, Sandground, Moore, Most and Carney, 1953), we found bladder lesions containing eggs in all 6 hamsters with *S. haematobium* infections from 33 to 67 weeks duration. This might be an indication that the migration of the worms from mesenteric to pelvic circulation in hamsters may be more consistent in older infections. With regard to the colonic egg lesions, the majority of the lesions were seen in the ascending and transverse colon, with few or no eggs found in the descending colon. The greatest concentration of eggs was at the hepatic flexure.

Viable eggs were first found in the feces 10 weeks after infection. The number of eggs passed by the infected hamsters increased for several weeks, and tended to decrease about 20 to 25 weeks after infection. We have observed the passage of viable eggs as long as 75 weeks after exposure.

TABLE 2.—*Recovery, maturity and distribution of* S. haematobium *in hamsters following percutaneous infection*

Duration of infection in weeks	Number cercariae per hamster	Number of hamsters	Total cercariae used	Number of worms recovered					Maturity of worms percentage		Distribution of worms percentage	
				Total	Mature		Immature					
					liver	mes.	liver	mes.	mature	immature	liver	mes.
6	200	6	1200	242			209	33		100	86	14
8	200	6	1200	234			138	96		100	59	41
9	200	7	1400	157	2	5	102	48	4	96	66	34
10	200	10	2000	418	210	206		2	99.5	0.5	50	50
12	200	10	2000	310	184	126			100		59	41
14	200	7	1400	203	67	136			100		33	67
16	200	6	1200	215	71	143			100		33	67
18	200	7	1400	257	80	177			100		31	69
20	200	6	1200	162	81	81			100		50	50
Total		65	13,000	2,198								
Average worms per hamster—33.8												
Percentage worm recovery—16.9%												

ALBINO RATS—A total of 5 albino rats was exposed percutaneously to 500 cercariae each after clipping the hair from the posterior half of the animal. The animals were autopsied from 6 through 14 weeks after exposure. Only 2 animals were found to be infected, and the worm yield was 1 and 2 immature worms respectively. The average worm recovery for the group was 0.6 worms per animal or 0.12% worm recovery.

GUINEA PIGS—A total of 14 guinea pigs was exposed percutaneously to 500 cercariae each after clipping the hair from the posterior half of the animal. The animals were autopsied at two-week intervals from 6 through 14 weeks after exposure. Only 2 of the animals were found infected. One immature worm was recovered from 1 animal, 10 weeks after exposure, and 10 immature worms were recovered from 1 animal autopsied 12 weeks after exposure. The average worm recovery for this series was 0.78 worms per animal or 0.16% of the cercariae employed for exposure.

RABBITS—A total of 7 rabbits was exposed by ear immersion to 1,500–11,000 cercariae per animal. The animals were sacrificed from 17 through 23 weeks after exposure. No worms were recovered from any of these rabbits.

SUMMARY AND CONCLUSIONS

The most satisfactory experimental animal for schistosome infections is one in which the worms are able to attain sexual maturity and deposit eggs in satisfactory locations so that they may be excreted in the feces. It is important to know the prepatent period of the infection and the expected worm recovery after the exposure of the animal to known numbers of cercariae.

In this study, albino mice, Syrian hamsters, albino rats, guinea pigs and rabbits were used. Only the mice and hamsters were found to be susceptible to infection with *S. haematobium*. Using 47 mice exposed percutaneously to 150 cercariae each, a recovery of 5.3 worms per mouse was obtained or 3.5% of the cercariae employed for exposure. In the 65 hamsters exposed percutaneously to 200 cercariae each, a recovery of 33.8 worms per hamster was obtained or 16.9% of the cercariae employed for exposure. Albino rats, guinea pigs and rabbits proved to be either completely or nearly completely refractory to infection with *S. haematobium*.

In the mice, the worms reached sexual maturity between 12 and 14 weeks after exposure, but more than 50% of the mature worms were found in the liver. In the hamsters, all of the worms reached maturity at 10 weeks after exposure, and a greater proportion of mature worms migrated to the mesenteric veins where eggs which were deposited had an opportunity to be excreted in the feces. In both mice and hamsters, intestinal egg lesions were essentially limited to the ileum and colon. In hamsters, the colonic egg lesions were most numerous in the ascending and transverse colon, with the greatest concentration of eggs found in the hepatic flexure. Few or no eggs were seen in the descending colon.

No bladder lesions were seen in any of the mice, indicating that there is no migration of worms from mesenteric to vesicular circulation up to 14 weeks in mice. Only a few hamsters exhibited bladder lesions, the earliest being observed at 14 weeks after exposure, indicating that the migration of worms to the pelvic circulation in hamsters is not consistent, at least up to 20 weeks.

Viable eggs were first found in the feces of infected mice 15 weeks after exposure. In hamsters viable eggs first appeared in the feces at 10 weeks. Egg passage in the mice seemed to be sporadic, while in the hamster the number of eggs passed increased for several weeks, and began to decline about 20 to 25 weeks after infection. Viable eggs were found in the feces of mice 62 weeks after infection and in hamsters 75 weeks after infection.

Considering the factors of worm recovery, prepatent period, migration of the mature worms to the mesenteric circulation, and passage of eggs in the feces, the hamster is the most suitable animal of those employed in these experiments.

References

Abdel-Azim, M. and Barlow, C. H. 1948 Studies on the development of *Bilharzia haematobium* and *Bilharzia mansoni* in experimentally infected mice (*Mus musculus*). 5th Annual Rep. Bilharz. Snail Destruct. Sec. 1946–1947, Ministry of Health Cairo. pp. 32–33.

Brumpt, E. 1928 L'homme est-il le seul semeur de germes dans le cas de la bilharziose vésicale? Bull. Acad. Méd. Paris, **100** (28) : 813–818.

Meleney, H. E., Sandground, J. H., Moore, D. V., Most, H., and Carney, B. H. 1953 The histopathology of experimental schistosomiasis II. Bisexual infections with *S. mansoni, S. japonicum* and *S. haematobium*. Am. J. Trop. Med. and Hyg. **2:** 883–913.

Moore, D. V., Yolles, T. K., and Meleney, H. E. 1949 A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni*. J. Parasit. **35:** 156–170.

———, Thillet, C. J., Carney, D. M., and Meleney, H. E. 1953 Experimental infection of *Bulinus truncatus* with *Schistosoma haematobium*. J. Parasit. **39:** 215–221.

Standen, O. D. 1949 Experimental schistosomiasis II. Maintenance of *Schistosoma mansoni* in the laboratory with some notes on experimental infection with *S. haematobium*. Ann. Trop, Med. and Parasit. **43:** 268–283.

Stunkard, H. W. 1946 Possible snail hosts of human schistosomes in the United States. J. Parasit. **32:** 539–552.

Watson, J. M., Abdel-Azim, M., and Halawani, A. 1948 Investigations on the anti-bilharzial action of miracil D. (nilodin). Trans. Roy. Soc. Trop. Med., and Hyg. **42:** 37–54.

Yolles, T. K., Moore, D. V., DeGiusti, D. L., Ripsom, C. L., and Meleney, H. E. 1947 A technique for the perfusion of laboratory animals for the recovery of schistosomes. J. Parasit. **33:** 419–426.

A NEW MYXOSPORIDIAN, *MYXOSOMA SQUAMALIS*, PARASITE OF SOME SALMONOID FISHES[1]

Edwin S. Iversen
Pacific Oceanic Fishery Investigations
U. S. Fish and Wildlife Service
Honolulu, T. H.

Introduction

For several years two commercial trout farms located near Seattle and one at Olympia, Washington experienced a considerable loss of rainbow trout (*Salmo gairdnerii* Richardson) due to a disease or diseases. Diseased fish examined in 1951 exhibited many tiny, milky-white pustules on their bodies (Figure 1.)

On occasion adult silver salmon (*Oncorhynchus kisutch* Walbaum) and chum salmon (*Oncorhynchus keta* Walbaum) show similar whitish pustules on the body surfaces. Infected silver salmon are apparently canned without removal of the in-

Received for publication, January 4, 1954.

[1] This is a portion of a thesis submitted in partial fulfillment of the degree of Master of Science to the School of Fisheries, University of Washington, Seattle.

tegument, but the pustules are not known to cause a reduction in the quality of the pack. The unappetizing appearance, however, may lower the value of this species when sold on the fresh-fish market.

Many species of myxosporidians have been reported from the integument of fishes. These differ from the present species in spore characteristics and usually in the specific site of infection. Two that possess the iodinophilous vacuole and differ in other spore characteristics, but have the ability to break down the scale substance have been reported.

Chakravarty (1939), working on *Myxobolus mrigalae* Charkravarty, present in *Cirrhina mrigala,* reported large cysts "on the scales of fish." He observed that "the fish scales seemed perforated after taking out the cysts. They are bounded by a fibrous inner layer which was surrounded by an outer layer of cells derived from the epidermis of the host."

Keysselitz (1908) named a new species *Myxobolus squamae,* which appears in the barbel, *Barbus fluviatilis.* The site of infection was the inner surface of the scales, in which an excavation is made. He noted that the myxosporidian bodies may be 1 to 8 on a scale and are enclosed in a strongly developed connective tissue envelope.

Shaw, Simms, and Muth (1934) very briefly mentioned the occurrence of a myxosporidian parasite "in the skin" of a silver salmon presumably from Oregon. They considered the parasite to be *Myxobolus squamae* Keysselitz. Judging from the photograph of an infected fish, the appearance of the skin is similar to that of fishes infected by the species here reported.

The site of infection of the sporulating trophozoite in the present species is within the scales; this, plus the observations on spore characteristics and its selection of the rainbow trout, the silver salmon, and the chum salmon as hosts, indicate that it has not been reported in the literature.

Zenker's solution and 10 percent formalin were the fixatives used for tissue to be sectioned and stained with Delafield's haematoxylin, counter-stained with alcoholic eosin. Nearly all sections were cut between 5 and 20 microns, the majority at 15 and 20 for minimum displacement of tissues.

The hanging-drop method was not necessary for fresh spore measurements, because in the usual smear technique, scale debris, present in all preparations, supported the coverglass so that no pressure was placed on the organisms. All drawings and measurements were made with the aid of a camera lucida. All measurements of fish are given in terms of total length, defined as the distance between the tip of the snout and the most posterior portion of the caudal fin. Terminology used in this study is that used by Kudo (1920, 1946).

EFFECT ON THE HOST

The extent of infection in some cases was considerable. A 12-cm. rainbow trout was covered with infected scales, so numerous that a count was impossible, but most individuals exhibited a weaker infection. The size of some of the infected fish from trout farms ranged from 9 cm. to 16 cm. Of 1,687 silver salmon examined at two canneries in Anacortes, Washington during the fall of 1952, 86 were infected or about 5.1 percent. One silver salmon, 65 cm. in length, had 140 infected scales on the right side and 147 on the left. Only 1 chum salmon in a small number examined was found infected.

Tissue sections were made from 3 rainbow trout, 2 silver salmon, and 1 chum salmon. In 1 rainbow trout, free from external trematodes, slight changes in the epidermis above infected scales were noticeable in sectioned material. A substantial reduction in numbers of mucous cells and a thinning of epithelial cells was evident (Figure 2). Nearly all the fish from this trout farm harbored a nematode, *Cucullanus truttae,* in the pyloric caeca.

In sections from all fish the connective tissue above the scales in the scale pockets showed various modifications. The fibroblasts became much more loosely arranged and appeared to be very delicate and spindle-shaped. Tissue preparations revealed that the margins of the scales not split by the parasite were modified. Usually, the free edge rolled beneath the scale which thus had a hook-like appearance.

On some infected scales a growth was noted which covered the entire inner side of the scale and possessed many nuclei of various shapes (Figure 3). This may be a pronounced hyperactivity of the host tissue in response to the parasite. However, that this is, in fact, wholly or partially modified host tissue and no portion of it is myxosporidian is highly uncertain. Occasionally spores were noted in this location, apparently occurring naturally and not displaced to this location by the technical procedures.

All infected fish revealed that the nuclei of the muscle cells were hypertrophied. They were spherical, spheroidal, or irregular in shape, and apparently were rich in chromatin as evidenced by their affinity for nuclear stain. Similar responses by host tissue to histozoic Myxosporidia have been noted many times in the past—Hahn (1915, 1917), Kudo (1926, 1929), and Nigrelli and Smith (1938).

No evidence was found that the disease under study was the sole cause of the death of any of the fish.

Myxosoma squamalis n. sp.

Trophozoite: The sporont can be easily obtained by teasing apart infected scales which do not show a mature infection. The fresh sporont is roughly circular to oblong in outline and may be monosporoblastic or disporoblastic (Figure 4). A typical sporont measured 19 microns, and the range is from 16 to 24 microns, based on only five measurements. The cytoplasm of both fresh and Giemsa-stained sporonts revealed numerous, small vacuoles.

Regarding the arrangement of the sporonts within the tumor, the usual reports mention that they are to be found mostly in the periphery of the trophozoite, and the center is occupied by free spores. In this case, examination of sections seems to indicate that sporonts may be present anywhere in the trophozoite within the infected scale. The size of the trophozoite varies with the size of the scale.

Spore: The spore is rounded to slightly oblong in front view, pyriform in side view (Figure 5, A and B), and lenticular in end view (Figure 5 C). The sutural ridge, which is without sutural markings of any kind is narrow, the sutural line indistinct. Closely bounding either side of the sutural ridge is a narrow parallel ridge that runs completely around the valve. This is readily noted in edge view. The shell valves, devoid of appendages, are relatively thin throughout and of equal size.

The convergent pyriform polar capsules are of approximately equal size and are located at the anterior end of the spore (Figure 5, A and D). The discharged polar filaments are of equal length (Figure 5 E). Polar filaments may be readily seen

TABLE 1.—*Dimensions of fresh spores*

Character	Mean (microns)	Standard deviation	Range (microns)	Number examined
Valves				
length	9.0	0.37	8.1–9.9	110
breadth	8.6	0.36	7.7–9.9	110
thickness	6.7	0.46	5.6–7.7	110
Polar capsule				
length	4.4	0.31	3.9–5.1	110
width	3.1	0.25	2.6–3.9	110
Polar filament				
length	35.2	2.21	30.9–38.3	50

coiled in the capsule in fresh material, but only with difficulty in preserved material. Four turns or windings are noted.

Despite rather prolonged exposure (1 week) to freshly-prepared Lugol's solution, no iodinophilous vacuole was found in spore smears made of fresh or formalin-preserved material. The single, granulated sporoplasm, when stained, reveals 2 nuclei. The dimensions of fresh spores mounted in natural fluids and measured at random are analyzed in Table 1.

Spores fixed in 10 percent formalin for a period of nearly 4 years, stained in Giemsa stain, and mounted in cedarwood oil, were measured. The analyses are given in Table 2.

The mean dimensions of stained spores, when compared with those of fresh spores, showed a reduction of 18 percent in valve length, 23 percent in breadth, 16 percent in width of polar capsule, and 19 percent in length of polar capsule.

Extrusion of filaments was noted in the present species, using spores kept on ice about 24 hours after the death of the silver salmon. Extrusion was stimulated by using the digestive juices of a freshly-killed rainbow trout 16.5 cm. in length. Five polar filaments were seen in the act of everting, but the amoebula was not seen to leave the shell valves. The filaments of a single spore did not evert simultaneously. When a filament everted, the spore would recoil in an opposite direction and spin rapidly at first then slowly until it came to a stop. Only a few revolutions were actually made in the medium of pus. The application of pressure to the coverslip also caused extrusion of filaments with regularity.

The form of the spore of the *Myxosoma* under study is believed to differ sufficiently from closely related species to be considered a new species, and the name *Myxosoma squamalis* n. sp. is suggested. Material upon which the description of the organism was based is in the collection of Dr. James E. Lynch at the School of Fisheries, University of Washington, Seattle 5, Washington.

DISCUSSION

No work was done to ascertain the viability of spores. A very interesting and worthwhile study on this species could be carried out with regard to the tolerance

TABLE 2.—*Dimensions of spores, formalin-fixed, Giemsa-stained*

Character	Mean (microns)	Standard deviation	Range (microns)	Number examined
Valves				
length	7.4	0.45	6.4–8.5	110
breadth	6.6	0.42	5.9–7.4	110
Polar capsule				
length	3.7	0.30	3.2–4.3	110
width	2.5	0.30	2.1–3.2	110

of spores to refrigeration, and the incidence of subsequent infection because of the practice of some hatchery men of using spawned-out silver salmon in the diet of hatchery fish. Difficulties involved in attempting to stain the sporoplasm of spores may give some indication of the ability of the organism to resist adverse conditions.

The size at which rainbow trout and silver salmon become infected, and the manner in which they become infected, are interesting problems on which no concrete information can be offered here. Judging from the small size of the infected rainbow trout, the life cycle can probably run to completion in about 7 months. In silver salmon it would seem logical that the young in fresh water receive the infection from spawning adults. If, however, the life cycle is shorter than 15 months in silver salmon then the infection must be picked up while the adults are in the salt-water stage of their existence. Whatever spores are not shed at that time are carried into streams, giving rainbow trout the opportunity to become infected.

Two of the trout farms may receive the infection from their water supply, a small river, which supports a run of silver salmon. The ocurrence of the disease at the trout farm near Olympia is conceivably due to the placing of infected fish in the ponds, since the water supply comes from a spring and is undoubtedly free from myxosporidians when it enters the ponds. The nature of the disease precludes suggestions as to therapeutic or prophylactic measures.

SUMMARY

A new species of myxosporidian parasite *Myxosoma squamalis,* from rainbow trout, silver salmon, and chum salmon, is described. This parasite in the trophozoite stage apparently attacks the surface of the scales of these fish and in some manner forces its way inside the scales and sporulates.

It is believed that as a result of the infection the epithelial layers and the fibroblasts of the scale pocket are modified in an area closely associated with an infected scale. That the parasite is a cause of mortality in the host fish is not known.

Measurements and a description of fresh spores are given, as well as measurements of spores fixed in formalin and stained in Giemsa. A substantial shrinkage was noted as a result of the fixing and staining. The size of the trophozoite varies with the size of the scale. Fresh sporonts are described, and these may contain 1 or 2 developing spores.

ACKNOWLEDGMENTS

The author wishes to express gratitude to Dr. J. E. Lynch, of the School of Fisheries, University of Washington, for direction and criticism throughout this work and for making available material from his collection. Indebtedness is acknowledged to Dr. R. R. Rucker of the Fish and Wildlife Service, for cooperation and helpful advice. Information concerning nematodes and their identification were kindly furnished by Dr. P. V. Gustafson of the University of Washington faculty. Mr. C. Schroeter and Mr. G. M. Southward gave generously of their time and effort to produce the photomicrographs and the photographs, respectively, presented here.

LITERATURE CITED

CHAKRAVARTY, MUKUNDAMURARI 1939 Studies on Myxosporidia from the fishes of Bengal, with a note on the myxosporidian infection in aquaria fishes. Arch. Protistenk. **92:** 169–178.

HAHN, C. W. 1915 Sporozoön parasites of certain fishes in the vicinity of Woods Hole, Massachusetts. Bull. U. S. Bur. Fish. **33:** 191–214.

———1917 On the sporozoön parasites of the fishes of Woods Hole and vicinity. I. Further observations on *Myxobolus musculi* from Fundulus. J. Parasit. **3**: 91–104.

Keysselitz, G. R. E. 1908 Die Entwicklung von *Myxobolus pfeifferi* Th., Teil I u II. Arch. Protistenk. **11**: 252–275, 276–308.

Kudo, R. R. 1920 Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Illinois Biol. Monog. **5**: 1–265 [239–503].

———1926 On *Myxosoma catostomi* Kudo 1923, a myxosporidian parasite of the sucker, *Catostomus commersonii*. Arch. Protistenk. **56**: 90–115.

———1929 Histozoic Myxosporidia found in fresh-water fishes of Illinois, U. S. A. Arch. Protistenk. **65**: 364–378.

———1946 Protozoology. 3d Ed. Pp. xiii + 778, 336 textfigs. Springfield, Ill. Charles C. Thomas.

Nigrelli, R. F. and G. M. Smith 1938 Tissue responses of *Cyprinodon variegatus* to the myxosporidian parasite, *Myxobolus lintoni Gurley*. Zoologica **23**: 195–202.

Shaw, J. N., B. T. Simms, and O. H. Muth 1934 Some diseases of Oregon fish and game and identification of parts of game animals. Station Bulletin (*322*). Oregon Agric. Exper. Station, *23* pp.

Plate I

Fig. 1. Photograph of a 12.4 cm. rainbow trout showing the typical appearance of a heavy infection within the scales.

Fig. 2. Photomicrograph of a longitudinal section of skin just anterior to dorsal fin of 15.3 cm. rainbow trout, showing the number of spores (S_1) within scale (S) and thinning of epidermis (E) with loss of mucus-secreting cells (M) above infected area. Melanophores (P), which appear as large black cells, may be seen in the dermis (D) and epidermis. 15 microns. ×129.

Fig. 3. Photomicrograph of transverse section of skin from belly of 15.3 cm. rainbow trout, showing spores (S_1) within scale (S) and what appears to be modified host tissue (T) beneath scale and above dermis (D). 5 microns. ×258.

Fig. 4. Disporoblastic sporont with nearly mature spores, Giemsa-stained. ×2475.

PLATE I

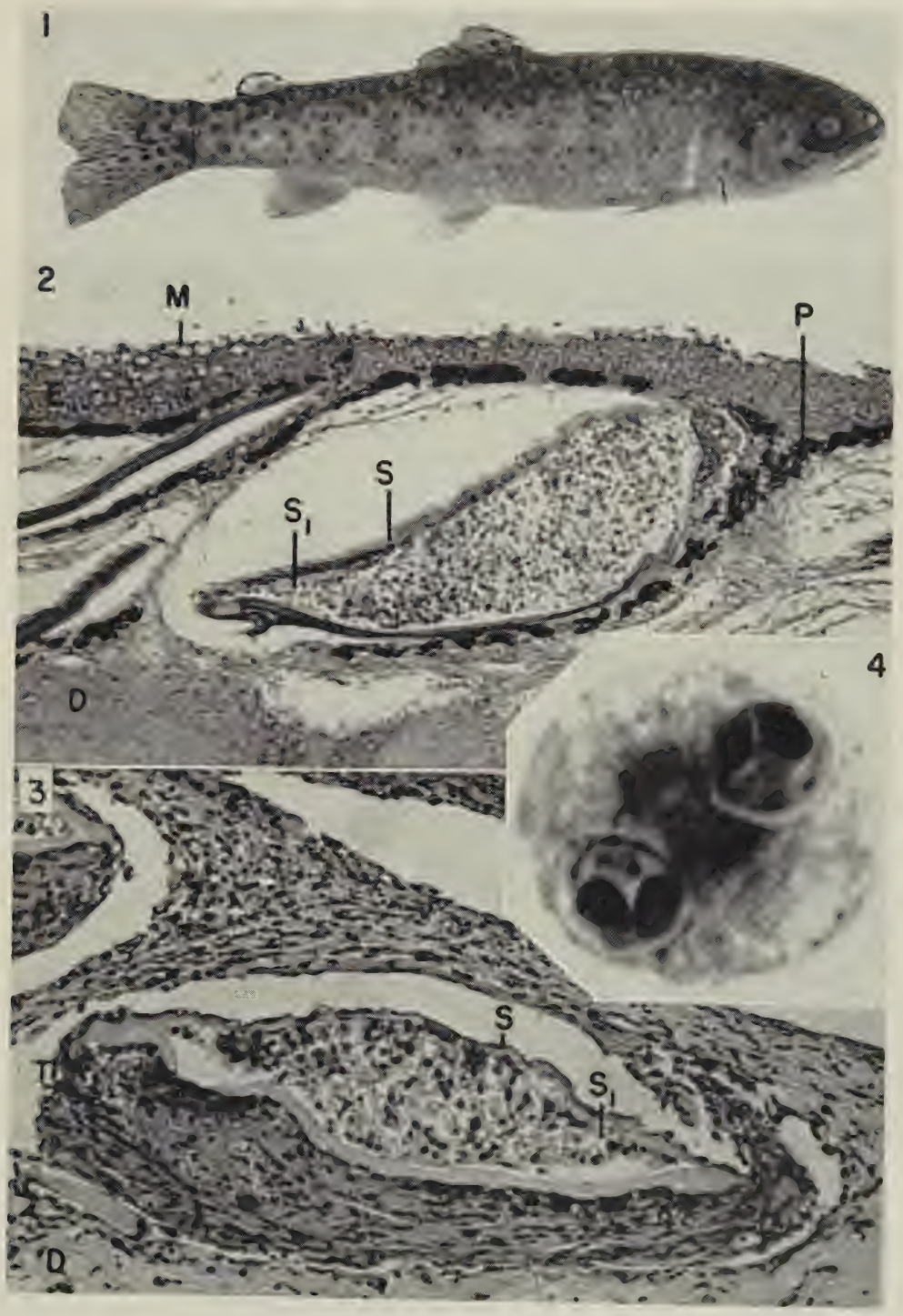


PLATE II

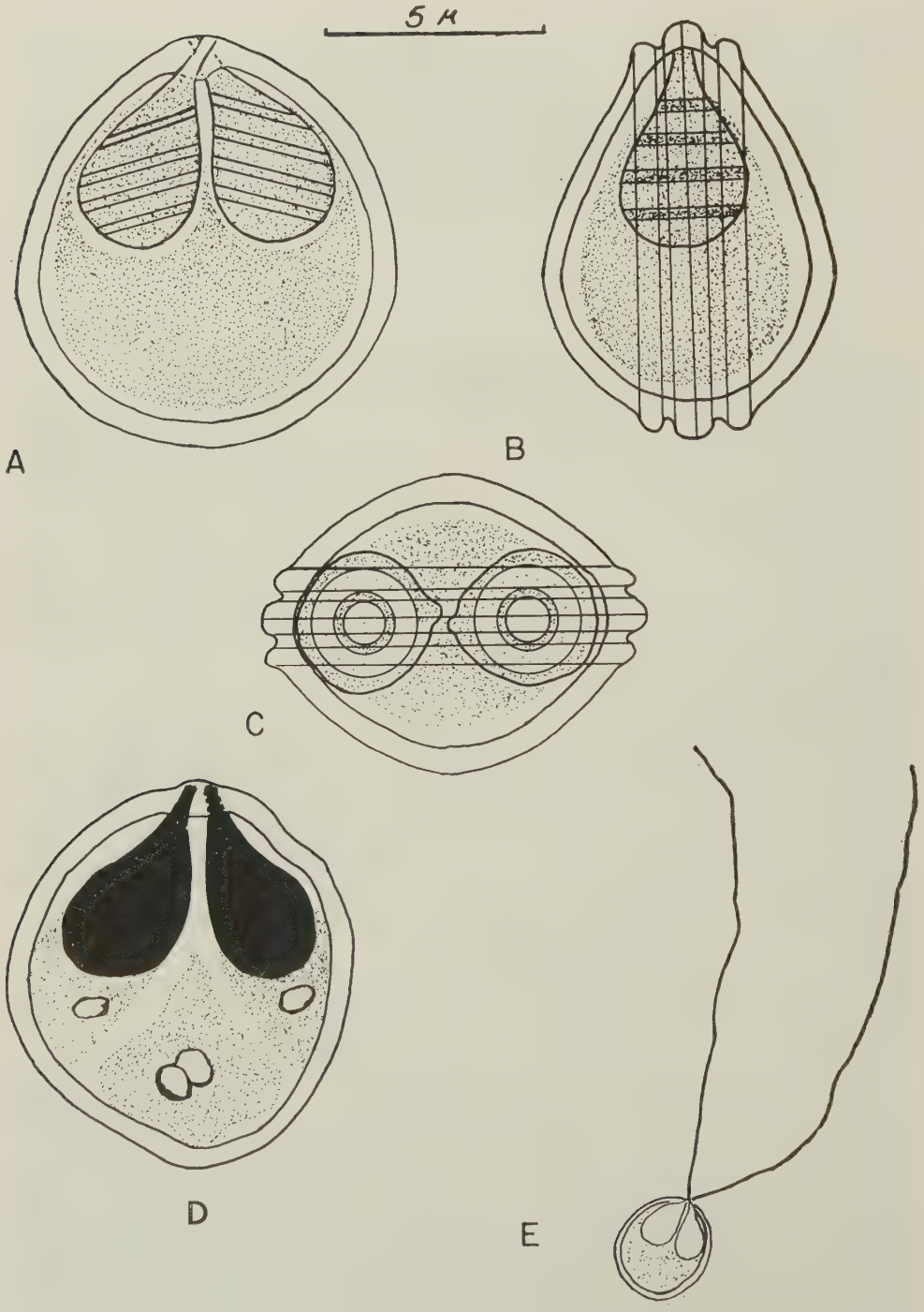


FIG. 5. Spores. (A) Front view, fresh spore, × 5644. (B) Side view, fresh spore, × 5644. (C) End view, fresh spore × 5644. (D) Front view, immature spore stained with Giemsa stain, × 4720. (E) Front view, fresh spore with polar filaments everted, × 1411.

THE PREDOMINANCE OF SINGLE-BROOD INFECTIONS IN HUMAN ASCARIASIS

RODNEY C. JUNG

Department of Tropical Medicine and Public Health
Tulane University of Louisiana
New Orleans, Louisiana

Ascariasis is acquired by the ingestion of soil containing infective eggs of *Ascaris lumbricoides*. In the United States the infection is primarily a childhood disease, Cort, *et al.*, 1930, Headlee, 1936, Beaver, 1952). It therefore appears probable that between the ages of 1 and 4 years. This is apparently due to the geophagous habit of children of this age rather than to the acquisition of an immunity as the child grows older, since adults are commonly infected in areas where the opportunity for ingestion of eggs exists. Not only can a history of dirt-eating be elicited in the case of most *Ascaris* infections but frequently the eggs of *Ascaris* can be recovered from the soil of the area in which the child is known to play (Brown, 1927, Cort, *et al.*, 1930, Headlee, 1936, Beaver, 1952). It therefore appears probable that during favorable seasons these children may ingest numbers of infective eggs almost daily. It is a matter of some interest, whether a large percentage of these eggs can develop on successive occasions and thus massive infections can be acquired, or whether massive infections result only from the ingestion of a large number of eggs at once or during a short period. For convenience worms derived from eggs ingested at about the same time will be referred to as a "brood."

Observations by Dr. Paul C. Beaver since 1947 and by the writer since 1948 on the pediatric wards of Charity Hospital, where there is about 20 percent prevaence of ascariasis, have suggested that with few exceptions infection with a single brood of *Ascaris* is the rule. The present report is intended to call attention to this fact, which, though probably recognized by many parasitologists and physicians, appears not to have been placed on record.

Ascaris lumbricoides were obtained from a total of 7 patients with massive infection either by medical treatment, or at surgery for removal of worms causing intestinal obstruction, or at autopsy. The worms were measured and their sexes determined.

Table I lists the manner in which the worms were acquired and the mean, median and range of lengths of worms in each case. The curves representing the frequency distribution of lengths, based on measurements of 50 or more worms, were all fairly symmetrical, with a single mode, and of limited spread. Worms of a single infection tended to be of one size, although there was considerable variation in the size of worms from patient to patient, depending presumably in part on the age of the infection. In case I the worms had barely become sexually differentiated. These data are indicative of a single brood of worms in the intestine.

These observations suggest that consistently there is but a single brood of intestinal *Ascaris*, even in the heavy infections. This phenomenon was previously observed in a single case by Otto (1931), who concluded that there is a rapid turnover in *Ascaris* infection resulting from the massive acquisition and spontaneous loss of worms. Although children with ascariasis spontaneously pass worms, (Otto,

Received for publication, January 15, 1954.

1930, Cort, Schapiro, Riley and Stoll, 1929), they do not often pass them in such numbers as to eradicate completely a bowel infection except over a period of several months. It is probable that meanwhile new broods of larvae fail to mature until the older worms are almost totally eliminated.

The inhibitory influence of successive infections with ascarids upon each other has been reported by Sadun (1948), who studied *Ascaridia galli* in the chicken. He found that the host response to a second infection may drive out the first, or may interfere with the development of the second. Beaver (1952) working with natural infections of *Ascaris lumbricoides* in the pig observed the consistent occurrence of worms of about the same size in the bowel of a single animal in all but 4 of 71 infected pigs. Although these results cannot be applied to pigs in general the writer is not aware of published evidence to the contrary based on actual measurements.

Sprent and Chen (1949) have shown that an infection with *Ascaris* larvae in experimental animals confers a type of immunity which tends to retain subsequent larval infections in the liver. The retarding influence is perhaps the tissue reaction,

TABLE 1.—*Lengths of worms in individual massive* Ascaris *infections*

Case	Manner collected	Total	Female worms Lengths (cm.)				Male worms Lengths (cm.)			
			Number	Mean	Median	Range	Number	Mean	Median	Range
I	Autopsy	570	278	20.7	18.5	9.0 – 30.0	165	13.7	13.5	7.0 – 21.5
II	Treatment	63	35	20.5	20.5	15.0 – 25.0	28	14.5	14.5	10.0 – 19.0
III	Surgery	93	44	22.5	22.0	19.0 – 27.0	49	15.7	16.0	9.0 – 19.0
IV	Treatment	52	29	19.7	18.5	13.0 – 24.5	23	13.7	14.0	11.5 – 19.0
V	Autopsy	246	151	19.0	19.0	13.0 – 25.5	95	14.0	14.0	10.5 – 18.0
VI	Treatment	56	33	21.4	21.0	15.0 – 33.5	23	11.8	14.0	12.0 – 22.0
VII	Treatment	129	95	11.3	10.5	7.0 – 14.0	34	8.9	9.0	6.0 – 12.0

which increases in intensity with successive infections (Sprent and Chen, *l.c.*, Kerr, 1938).

The fate of *Ascaris* larvae entering the body during an intestinal infection has not been studied. They may complete their migration and then be eliminated without maturing, may become ensnared as in Sprent's reinfection studies in abnormal hosts or may perhaps persist for a time but with reduced activity due to host tissue reaction. These *Ascaris* larvae may thus be capable of producing a disease similar to the visceral larva migrans produced by *Toxocara* (Beaver, Snyder, Carrera, Dent and Lafferty, 1952), a potentiality which is, however, limited by the relatively greater susceptibility of *Ascaris* to encapsulation and destruction by the host (Hoeppli, Feng and Li, 1949).

SUMMARY

In 7 cases of heavy *Ascaris lumbricoides* infection in human beings the worms were all collected and measured. The sizes of worms in each infection indicated that the worms were of about the same age. This suggests that a pre-existent intestinal infection with *Ascaris* may prevent the development of worms entering the body subsequently.

REFERENCES

BEAVER, P. C. 1952 Observations on the epidemiology of ascariasis in a region of high hookworm endemicity. J. Parasit. **38**: 445–453.

———, SNYDER, C. H., CARRERA, G. M., DENT, J. H. AND LAFFERTY, J. W. 1952 Chronic eosinophilia due to visceral larva migrans. Pediatrics **9**: 7–19.

BROWN, H. W. 1927 Human ascaris as a household infection. J. Parasit. **13**: 206–212.

CORT, W. W. 1931 Recent investigations on the epidemiology of human ascariasis. J. Parasit. **17**: 121–144.

———, OTTO, G. F. AND SPINDLER, L. A. 1930 Investigations on *Ascaris lumbricoides* and the associated intestinal helminths of man in southwestern Virginia. Am. J. Hyg. **11**: 1–55.

———, SCHAPIRO, L., RILEY, W. A. AND STOLL, N. R. 1929 A study of the influence of the rainy season on the level of helminth infestations in a Panama village. Am. J. Hyg. **10**: 626–634.

HEADLEE, W. H. 1936 The epidemiology of human ascariasis in the metropolitan area of New Orleans, Louisiana. Am. J. Hyg. **24**: 479–521.

HOEPPLI, R., FENG, L. C. AND LI, F. 1949 Histological reactions in liver of mice due to larvae of different *Ascaris* species. Peking Nat. Hist. Bull. **18**: 119–132.

KERR, K. B. 1938 The cellular response in acquired resistance in guinea pigs to an infection with pig ascaris. Am. J. Hyg. **27**: 28–51.

OTTO, G. F. 1930 *Ascaris lumbricoides.* Treatment, loss of worms and reinfestation. J. Am. Med. Ass. **95**: 194–196.

———1931 Massive acquisition of *Ascaris* by young child. J. Parasit. **17**: 233.

SADUN, E. H. 1948 Resistance induced in chickens by infections with the nematode, *Ascaridia galli.* Am. J. Hyg. **47**: 282–289.

SPRENT, J. F. A. AND CHEN, H. H. 1949 Immunological studies in mice infected with the larvae of *Ascaris lumbricoides.* I. Criteria of immunity and immunizing effect of isolated worm tissues. J. Infect. Dis. **84**: 111–124.

A NEW CHIGGER MITE (*GENUS EUSCHÖNGASTIA*) FROM THE CENTRAL UNITED STATES[1, 2]

LOUIS J. LIPOVSKY AND RICHARD B. LOOMIS

Studies of the chigger fauna of Kansas have revealed a species of chigger mite of the genus *Euschöngastia* described below, which is distinct from, but closely related to, the bat chigger, *Euschöngastia pipistrelli* Brennan, 1947. The terminology used throughout this paper is that of Wharton, *et al.,* 1951. All measurements are in microns.

We wish to thank Mr. Robert B. Finley, Jr., Mr. J. Knox Jones, Jr. and Mr. Ervin H. Kardos for aid in obtaining larvae and their hosts. Thanks are also extended to Mr. D. A. Crossley, Jr. and Dr. Charles D. Michener of the University of Kansas for reading the manuscript.

Euschöngastia jonesi[3] new species

[3] Named for Mr. J. Knox Jones, Jr., formerly at the University of Kansas, who caught the host from which the type series was obtained, and who has aided in the collection of many other chiggers and their hosts.

Diagnosis.—Closely related to *Euschöngastia pipistrelli* in having 1 tibiala III; 2 genualae I; subterminala and parasubterminala I; scutum twice as wide as long, with long posterolateral setae; palpal claw with 5 prongs; and lateral palpal tibial seta nude. It differs from the above mentioned species in having the galeal seta branched; sensilla clavate, gradually tapering distal part with a few large setules; and distinct eyes 2/2.

Received for publication, January 15, 1954.

[1] The studies upon which this paper is based were conducted under a contract, N6 ori 220 Task Order 2, between the University of Kansas and the Office of Naval Research.

[2] Contribution No. 864 of the Department of Entomology, University of Kansas.

Description of larva.—Body: Holotype (unengorged), 246 by 238 (engorged 802 by 483); white in life. Eyes, 2/2, pink in life, anterior slightly larger; length of both on 1 side 18, ocular plate faint.

Dorsal setal formula 2-13-13-13-14-10, total 64 (some paratypes with 14 setae in first and second rows); humeral seta measures 72, anterior dorsal seta 68, posterior dorsal seta 56. Ventral setal formula approximately 2-2-12-15-10-10-11-10-2, total 74; anterior sternal seta measures 54, posterior sternal seta 40, anterior ventral seta 25, posterior ventral seta 42. Total body setae approximately 138.

Scutum: Width more than twice the length, punctae small and scattered, posterior margin slightly rounded with a slight central concavity, a crescent-shaped ridge over each sensillary base, not meeting in center; bases of anterolateral and posterolateral setae situated on slight scutal projections, PL's long, AM and AL's approximately the same length, sensillary bases posterior to bases of the PL's. Sensilla clavate, tapering distally to a point, setules scattered, larger and decreasing in number distally. Scutal measurement of holotype: AW—67, PW—92, SB—34, ASB—31, PSB—12, AP—24, AM—49, AL—48, PL—83, S—45, (width 11). Average and extremes of 11 larvae both unengorged and engorged, 5 from Cowley, 2 from Douglas, and 4 from Barber County, Kansas; AW—67 (63–73), PW—92 (83–106), SB—33 (28–37), ASB—30 (26–33), PSB—12 (11–14), AP—24 (21–28), AM—45 (43–49), AL—47.5 (43–53), PL—90 (83–103), S—42 (41–46).

Gnathosoma: Chelicera blade long and stout, one tricuspid dorsal cap; base punctate. Galeal seta with 3 branches. Capitular sternum punctate, with a single pair of branched setae. Palpal femur and genu each with 1 branched seta, tibia with branched dorsal and ventral setae and nude or single-branched lateral seta; tarsus with a basal spur (10), and 7 branched setae. Palpal claw with 5 prongs, 2 short prongs and 3 long prongs of approximately equal length (see fig. 1).

Legs: Leg I coxa, trochanter and basifemur each with 1 branched seta; telofemur with 5 branched setae; genu with 4 branched setae, 2 genualae and a microgenuala; tibia with 7 branched setae, 2 tibialae and a microtibiala; tarsus with approximately 20 branched setae, a spur (18), microspur, subterminala, parasubterminala, and pretarsala. Leg II coxa and trochanter each with 1 branched seta; basifemur with 2 branched setae; telofemur with 4 branched setae; genu with 3 branched setae and a genuala; tibia with 6 branched setae and 2 tibialae; tarsus with 17 branched setae, spur (18), microspur, and a pretarsala. Leg III coxa and trochanter each with 1 branched seta; basifemur with 2 branched setae; telofemur with 3 branched setae; genu with 3 branched setae and a genuala; tibia with 6 branched setae and a tibiala; tarsus with approximately 15 branched setae. Setules reduced in number on setae of distal segments of all legs. All leg segments with scattered punctae.

Remarks.—Euschöngastia jonesi n. sp. has been taken on bats but never in the same locality as *E. pipistrelli.* The type host was captured at night in a small limestone cave. All of the hosts were taken in rocky habitats, limestone occurring in the east, sandstone and gypsum in the west.

The larvae were found in and on the ears of the hosts. The type larvae were scattered over the face and ears of the white-footed mouse.

This species has been reared to the nymphal stage by one of us (Lipovsky) from larvae taken in Barber County, and by Mr. D. A. Crossley, Jr., from larvae taken with the type series. The latter nymphs were individually correlated with cast larval skins.

Types.—Larvae: Holotype, slide no. 6930 and 25 paratypes, nos. 6931–55, Snow Entomological Museum, University of Kansas, from 2 miles east of Rock (14 miles north of Winfield), Cowley County, Kansas, taken from a white-footed mouse, *Peromyscus leucopus* (Rafinesque), field number RL530307-5, obtained on March 7, 1953 by J. Knox Jones, Jr. and Olin L. Webb.

Paratypes will be sent to the United States National Museum; Rocky Mountain Laboratory; British Museum (Natural History); Dr. George W. Wharton; The South Australian Museum, Adelaide; Dr. Charles D. Radford; the Museum National d'Histoire Naturelle, Paris, France; Army Medical Service Graduate School, Washington, D. C. and Dr. J. R. Audy, Institute for Medical Research, Kuala Lumpur, Malaya.

Additional specimens examined.—Total, 69 larvae, as follows. KANSAS. *Douglas County*: 5 mi. N, 1 mi. E Lawrence, Univ. Kansas Nat. Hist. Reservation, *Cryptotis parva,* Oct. 16, 1951, KU 6964–75 and chigger sampler, Nov. 9, 1951, KU 6976; 5 mi. S, 5 mi. W Lawrence, *Peromyscus leucopus,* April 28, 1952, KU 6977–78 (total 4 specimens); 2 mi. S, 2 mi. W Pleasant Grove, *Sylvilagus floridanus,* Dec. 4, 1948, KU 6979, Dec. 29, 1948, KU 6980–81, Jan. 29, 1949, KU 6982. *Cowley County*: 2 mi. E Rock, *Peromyscus leucopus,* March 7, 1953, KU 6955–63 (total 18). *Barber County*: 5 mi. S Sun City, *Peromyscus maniculatus,* April 12, 1949, KU 6984–89; 4 mi. S Aetna, *Neotoma micropus,* April 11, 1949, KU 6983, *Myotis velifer,* April 13, 1950, KU 6990–91, *Peromyscus maniculatus,* Oct. 7, 1951, KU 6992–98. OKLAHOMA. *Woods County*: 6 mi. S, 2 mi. W Aetna, Kansas, Merrihew Cave, *Myotis velifer,* Oct. 6, 1951, KU

6992–7002. MISSOURI. *Lawrence County*: 0.5 mi. S Verona, *Peromyscus leucopus,* Sept. 6, 1947, KU 7003–06.

SUMMARY

A new species of chigger mites, *Euschöngastia jonesi,* is described from various localities in Kansas, Oklahoma and Missouri.

Literature Cited

Brennan, J. M. 1947. New species of chiggers (Acarina, Trombiculidae) from bats of the Nearctic Region. J. Parasit., **33**: 245–252.

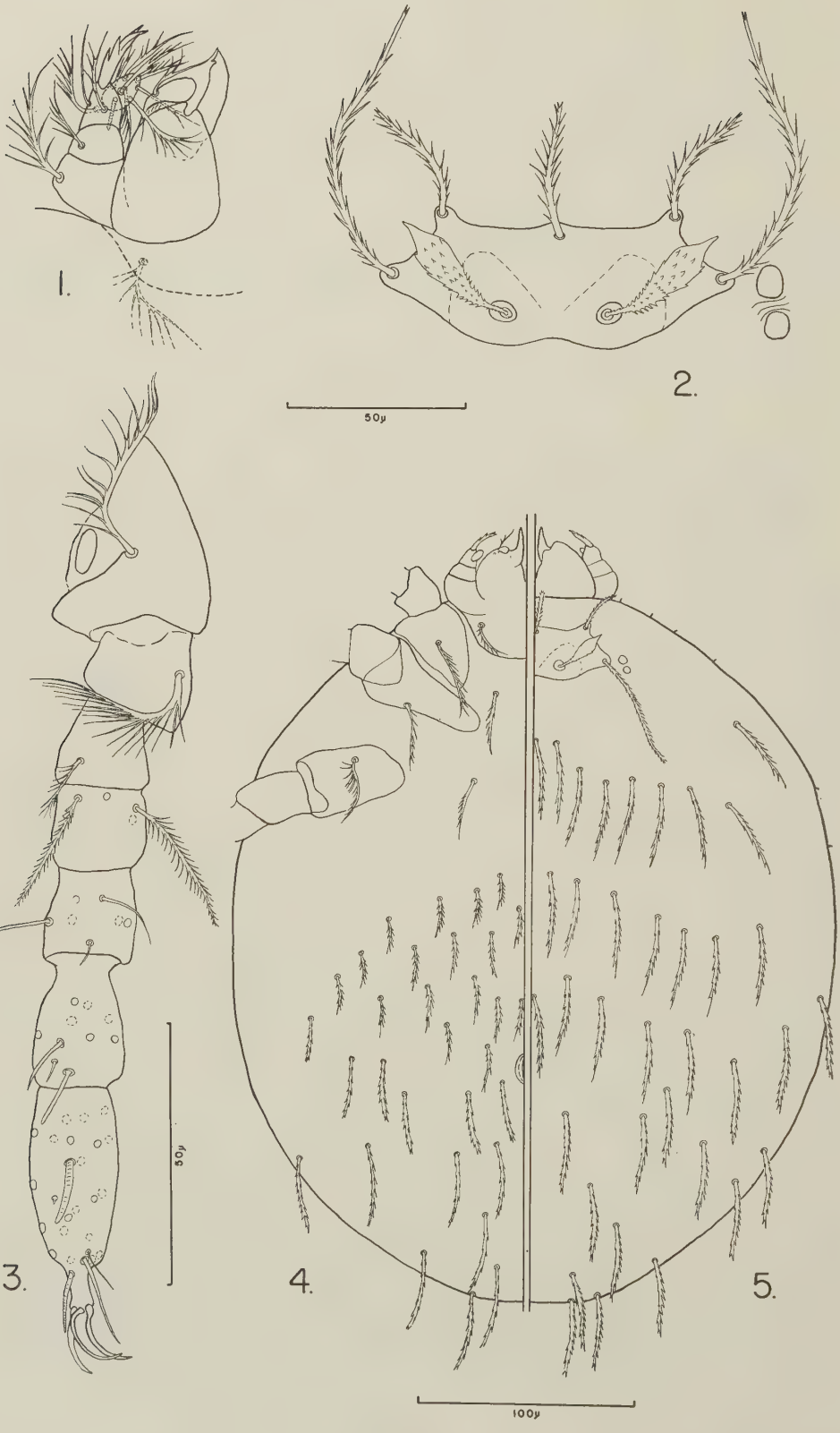
Wharton, G. W., D. W. Jenkins, J. M. Brennan, H. S. Fuller, G. M. Kohls, and C. B. Philip. 1951. The terminology and classification of trombiculid mites (Acarina, Trombiculidae). J. Parasit. **37**: 13–31.

Explanation of Plate

Euschongastia jonesi new species

Fig. 1. Dorsal aspect of gnathosoma.
Fig. 2. Scutum and eyes.
Fig. 3. Leg I, showing all nude setae.
Fig. 4. Ventral aspect of body.
Fig. 5. Dorsal aspect of body.

PLATE I



EXOGENOUS PROLIFERATION IN A LARVAL TAENIID (CESTODA: CYCLOPHYLLIDEA) OBTAINED FROM THE BODY CAVITY OF PERUVIAN RODENTS

MARIETTA VOGE

Department of Infectious Diseases, School of Medicine
University of California, Los Angeles

The occurrence of asexual larval multiplication in cestodes has been reported by various authors, and in recent years notably by Dollfus (1938, 1951). While endogenous proliferation occurs normally among members of the family Taeniidae, exogenous multiplication is less frequent and may in some instances represent an abnormal development (Kuntz, 1943).

The evidence presented below indicates that exogenous larval proliferation may be a part of the normal development of some taeniids. The description which follows is based on specimens obtained from the body cavity of three *Ctenomys peruanus* collected December 17, 1951, and February 5, 1952, at Pampa Queullecota, 13,000 ft., 60 km. S. Ilave, Department of Puno, Peru; from one *Phyllotis osilae* collected February 9, 1952, 15 km. W. Puno, 13,000 ft., Department of Puno, Peru; and from one *Chinchillula sahamae* collected January, 1952, 5 km. E. Lago Suche, 14,600 ft., Department of Moquegua, Peru.

I am very grateful to Dr. and Mrs. Oliver P. Pearson, Museum of Vertebrate Zoology, University of California, Berkeley, for collecting these interesting specimens for me. Thanks are due to Mr. Zane Price, Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, for the photograph in Plate I.

DESCRIPTION

The larvae from the three rodent hosts are fairly uniform in external appearance. Individual cysticerci vary from 9 to 15 mm. in length, and from 3 to 4 mm. in width; contracted specimens are usually less than 9 mm. long. The scolices are invaginated. At the posterior end, each cysticercus bears a slender stalk of variable length. In some groups of larvae, the stalk is confluent with a bladder from which the individual cysticerci proliferate; in other groups the point of attachment consists of a mass of dense tissue (Fig. a). The number of cysticerci originating from each bladder, varied from nine to 20 in presumably full grown specimens. Figure a illustrates such a group in which some of the larvae are contracted and others relaxed; all cysticerci are apparently derived from a single bladder.

A few specimens from *Chinchillula sahamae* suggest that the first step in the development of the group is the growth of one cysticercus which bears a relatively narrow and transparent bladder at its posterior end; from this bladder additional larvae proliferate. In such relatively "young" larval groups one may see individual cysticerci varying in length from 1 to 10 mm. or more; a bladder-like structure is always present. In other groups which consist of individuals uniform in size, the bladder is usually much smaller or absent entirely. In its place, there is a variable amount of stringy and hardened tissue (Fig. a).

Hook size and number vary considerably, depending on the stage of development of the specimen examined. In apparently fully grown cysticerci, the number of

Received for publication, November 13, 1953.

hooks was 40–44; of these, 20 or more measured 120–140 microns (Fig. c), and alternated in position with a similar number of larger hooks measuring 190–220 microns in length (Fig. b).

I have previously reported different data for hook size and number of these cysticerci (Voge, 1953). It appears from a more thorough investigation of the material, that the data in my earlier report were obtained from larvae which were not fully grown.

DISCUSSION

In external appearance, the cysticerci from the Peruvian hosts resemble the description given by Dollfus (1951) of taeniid larvae recovered from the pleural cavity of *Gerbillus pyramidum* in Africa. The Peruvian material differs from that described by Dollfus in the number, size and shape of the hooks, which are much smaller and less numerous in my specimens. Dollfus (1951) includes a table of data recorded by other authors who described exogenous proliferation in taeniid larvae thought to be *Taenia taeniaeformis* (Bloch, 1780). In all of these, the hooks are considerably larger than they are in my specimens. Schwartz (1924) described and illustrated an exogenously proliferating cysticercus, *Taenia twitchelli,* from the lungs of a porcupine but his species bears little resemblance to the Peruvian specimens. It should be stressed that hook size varies with the stage of development of the larva; comparisons are of little value unless based on hooks which have reached their maximal development within the cysticerci. External structure, particularly the shape of individual cysticerci and the mode of branching, might in some instances be a more reliable criterion for differentiation.

Specific identification of the Peruvian cysticerci described herein, cannot be made until more is known about the adult taeniids in the High Andes. Further indication that exogenous proliferation is a regular occurrence in this species is evident in the observations of Dr. and Mrs. Pearson, who recorded these larvae from at least six *Ctenomys peruanus* and noted them in several other individuals of the same host species.

SUMMARY

Exogenous proliferation is described in taeniid larvae obtained from the body cavity of three species of Peruvian rodents. This type of asexual, larval multiplication was observed in all specimens studied and is therefore thought to be a normal part of the development of this cestode. In view of our scanty knowledge of adult taeniid cestodes in Peru, specific identification or description is at present not desirable.

REFERENCES

DOLLFUS, R. P. 1938 Sur un *Cysticercus fasciolaris* Rudolphi tératologique (polycéphale). Ann. Parasit. hum. comp. **16** (2) : 133–141.

———1951 Cystique polycephale de Taenia chez une gerbille. Ann. Parasit. hum. comp. **26** (4) : 274–278.

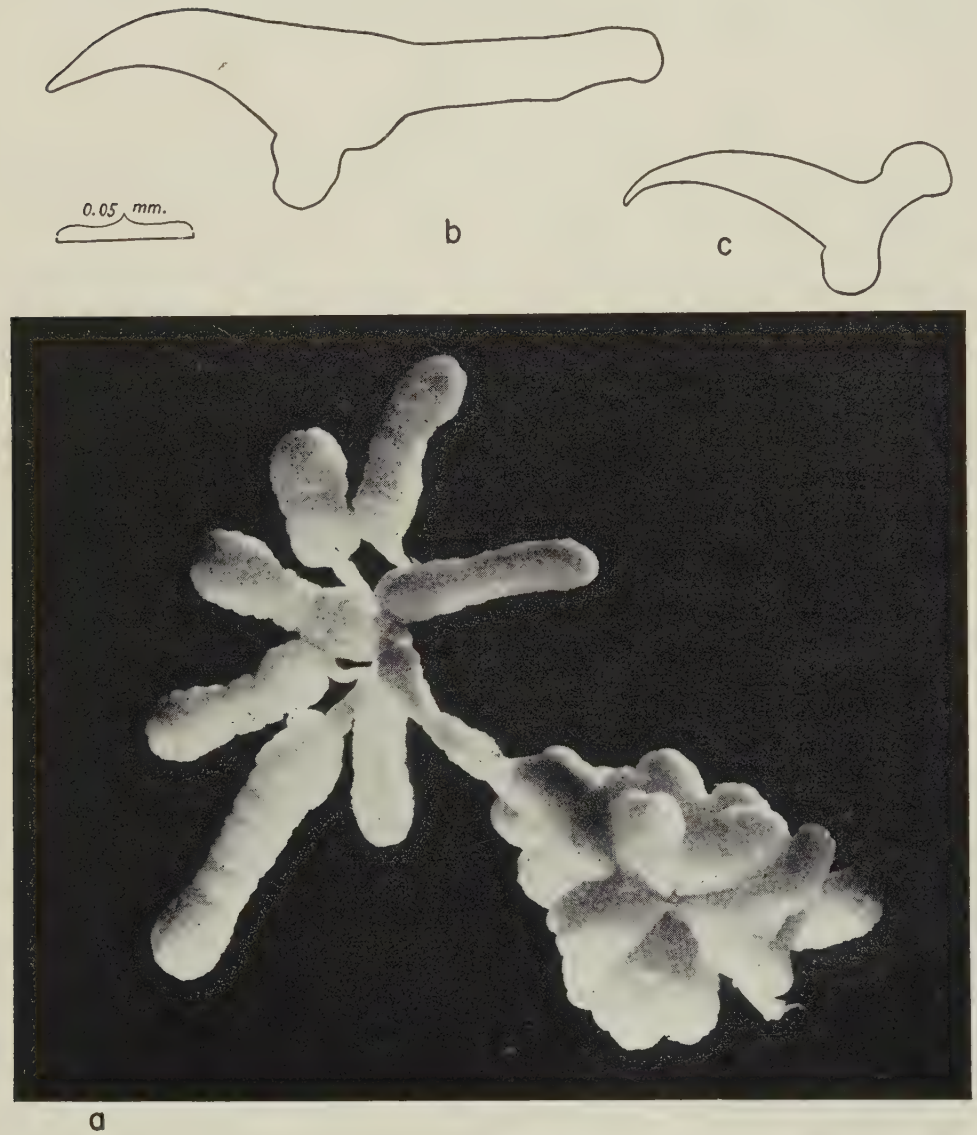
——— AND CHABAUD, A. G. 1951 Miscellanea helminthologica maroccana II. Cystique polycéphale chez un *Meriones shawi* (G. L. Duvernoy, in C. A. Rozet, 1833). Arch. Inst. Pasteur Maroc **4** (3) : 230–235.

KUNTZ, R. E. 1943 Cysticercus of *Taenia taeniaeformis* with two strobilae. J. Parasit. **29:** Nat. Mus. (2561) **66,** Art. 24, 1–4. 424–425.

SCHWARTZ, B. 1924 A new proliferating larval tapeworm from a porcupine. Proc. U. S.

VOGE, M. 1953 Exogenous proliferation in a larval taeniid (Cestoda: Cyclophyllidea) from the body cavity of Peruvian rodents. J. Parasit. **39:** 32. (Abstract.)

PLATE I



EXPLANATION OF PLATE

Drawing made with the aid of a camera lucida.

PLATE I

FIG. a. Photograph of a group of cysticerci from the body cavity of *Ctenomys peruanus;* magnification a little over 3× original size.

FIG. b. Large hook.

FIG. c. Small hook.

SOME FACTORS AFFECTING THE INTENSITY OF *EUHAPLORCHIS CALIFORNIENSIS* (TREMATODA: HETEROPHYIDAE) INFECTIONS IN CHICKS[1]

LOIS W. WONG[2]
Department of Zoology, University of Southern California

The purpose of this investigation was to determine factors affecting the intensity of infection of *Euhaplorchis californiensis* in chicks by variation in diet and period of starvation of the host.

Infections of *Euhaplorchis californiensis* have been experimentally established in the small intestine of chicks (Martin, 1950); in cats (Bamberger and Martin, 1951); in monkeys (Bamberger and Martin, unpublished); and in lizards and bats (Meniskes and Martin, unpublished). The life cycle of the trematode was described by Martin (1950).

Few previous reports have compared the number of metacercariae fed and the number of adult flukes developed to maturity in the definitive host. In a study of the resistance of metacercariae (*Haplorchis taihokui*) to various methods of preparing raw fish for human consumption in the Philippine Islands, Garcia (1936) recovered 22 to 23 flukes for each 30 metacercariae about 2 weeks after they were fed to control mice. Bamberger and Martin (1951) recovered 2,511 (about 12%) *Euhaplorchis californiensis* adults from a control cat fed fish brains containing approximately 20,000 metacercariae and sacrificed 10 days after the initial feeding.

Some studies have been made to determine the effects of the host's diet on resistance to cestode and nematode parasites, but no reports on trematodes have been found. Zimmerman, Vincent and Ackert (1926), Ackert, Fisher and Zimmerman (1927), Ackert and Spindler (1929) and Ackert and Nolf (1931) showed that a deficiency in certain vitamins lowered the resistance of chickens to the nematode, *Ascaridia lineata*. Clapham (1933, 1934) showed that vitamin A increased the resistance of chicks to *Heterakis gallinae* while a diet deficient in mineral content lowered resistance to that parasite.

Ackert and Beach (1933), Ackert, Branson and Ameel (1944), Ackert (1947), Riedel (1946) and Riedel and Ackert (1950) found that inclusion of skim milk in the diet increased the resistance of the chicks to the nematode, *Ascaridia lineata*. Becker (1933) found that a milk diet caused the cessation of egg production of the cestode, *Hymenolepis nana*, in the rat but did not kill the worms.

Starvation of the host also affects helminths. Levine (1938) found that there was a marked decrease in proglottid production of *Davainea proglottina*, when the chick hosts were fed rations with little or no food value. No decrease in egg production by *Hymenolepis diminuta* was noted by Hager (1941) who fed the rat host a partial starvation diet by reducing the food intake to one-third of the normal

Received for publication, November 2, 1953.

[1] Part of a dissertation submitted to the University of Southern California in partial fulfillment of requirements for the degree of Doctor of Philosophy. The writer is indebted to Dr. W. E. Martin, University of Southern California, for his guidance and advice throughout the investigation.

[2] Now at the Department of Parasitology, School of Tropical and Preventive Medicine, Loma Linda, California.

The smallest number of flukes recovered was from the chicks which had access to food at all times. The greatest number of worms was recovered from chicks which had no access to food for 5 hours after the feeding of metacercariae. The average number of flukes remained approximately the same in oatmeal-fed chicks that were starved for 5 or 10 hours after the feeding of metacercariae, and decreased in chicks which were fasted for 15 hours. The number of worms recovered from chicks fasted 5 hours before and 5 hours after the feeding of metacercariae was smaller than the number recovered from chicks fasted for only 5 hours after ingesting the cysts. The number of flukes recovered from chicks starved for 5 hours before the feeding of metacercariae was smaller than the number recovered from those starved for 5 hours after ingesting the cysts.

TABLE 1.—*Difference in intensity of fluke infection in chicks due to different food and varying periods of fasting. Each chick was given 500 metacercariae*

	FEED														
	Group I All Purpose Chick Feed					Group II Oatmeal					Group III Powdered Milk				
			Flukes recovered					Flukes recovered					Flukes recovered		
Fasting Condition	No. of chicks	Age in days	Total no.	%	Av. no. per chick	No. of chicks	Age in days	Total no.	%	Av. no. per chick	No. of chicks	Age in days	Total no.	%	Av. no. per chick
Class A No fasting	6	2–10	3	0.1	0.5	6	2–10	20	0.6	3.3					
Class B Fasting 5 hrs. before feeding of metacercariae	6	2– 7	3	0.1	0.5	6	2– 6	143	4.7	23.8					
Class C Fasting 5 hrs. after feeding of metacercariae	6	2– 5	17	0.6	2.8	6	2– 6	650	21.7	108.3	6	3–9	64	2.1	10.7
Class D Fasting 5 hrs. before and 5 hrs. after feeding of metacercariae	6	4– 7	11	0.4	1.8	6	3–10	307	10.2	51.2					
Class E Fasting 10 hrs. after feeding of metacercariae						6	2– 3	616	20.5	102.7					
Class F Fasting 15 hrs. after feeding of metacercariae						6	2– 3	372	12.4	62.0					

Of the 6 chicks fed only powdered milk and fasted for 5 hours after the feeding of 500 metacercariae each, 4 to 29 flukes were recovered from 4 chicks and none from the other 2. This count is much lower than for chicks fed oatmeal and is a little higher than for those fed chick feed.

The content of food in the chick gut apparently interfered with excystment or with attachment of flukes to the host. Chicks which had free access to food (oatmeal or chick feed) on the day that the metacercariae were ingested yielded few flukes. Starving the host for 5 to 10 hours after receiving metacercariae probably allowed the worms to attach themselves at their usual position in the lower part of the intestine and thus increased the number of parasites recovered from the chicks.

The present experiment indicates that a deficient diet and fasting of the host for a short period after the feeding of metacercariae are factors enabling *E. californiensis* to develop in greater numbers in an unnatural host such as the chick.

All adult flukes were recovered from the last 10 inches of the intestine, and most of them from the last 4 inches of this segment; but some were occasionally recovered from the ceca and, less frequently, the rectum. Of the total of 3,259 flukes recovered from 113 chicks fed 500 metacercariae each; 5 per cent were found in the rectum, 10.5 per cent in the ceca, 28 per cent in the segment 0 to 2 inches anterior to the rectum, 35 per cent in the segment 2 to 4 inches anterior to the rectum, 18.4 per cent in the segment 4 to 6 inches anterior to the rectum, 2.9 per cent in the 6 to 8 inch segment, 0.2 per cent in the 8 to 10 inch segment anterior to the rectum, and none anterior to this last segment. See figure 1.

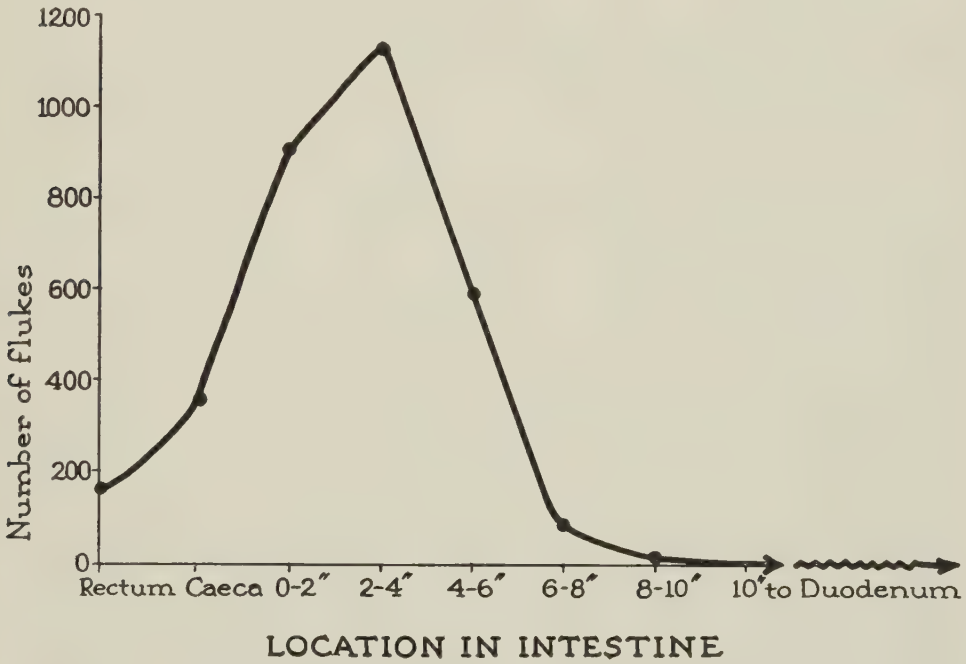


FIG. 1. Relationship of the number of flukes to their location in the rectum, caeca, and 2-inch segments of intestine forward.

Five hours after 1 chick was fed 500 metacercariae, 82 per cent of the total number of flukes present occurred in the last 4 inches of the intestine anterior to the rectum. In 2 other chicks fed 20 infected fish brains each and sacrificed 5 hours later, the majority of flukes recovered also occurred in the 4 inches of intestine anterior to the rectum. Thus, the majority of the worms became located in the posterior portion of the intestine very early after an infective feeding of the host.

SUMMARY

1. A large number of adult *Euhaplorchis californiensis* were recovered from chicks which were fed oatmeal than from those fed an all-purpose chick feed.

2. Allowing the birds to have access to food at all times rendered conditions less favorable for the development of *Euhaplorchis californiensis*.

3. Chicks fed an oatmeal diet and fasted for 5 to 10 hours after the feeding of metacercariae showed the heaviest infections.

4. Fewer adult flukes were found in milk-fed chicks than those fed oatmeal.

5. Most of the worms were found in the last 4 inches of intestine.

Literature Cited

Ackert, J. E. 1947 Soybean oil meal supplement effective in maintaining host resistance to ascarids. Revista *Kuba* Med. Trop. Parasit. **3**: 92–94.

——— and Beach, T. D. 1933 Resistance of chickens to the nematode, *Ascaridia lineata*, affected by dietary supplements. Tr. Am. Micr. Soc. **52**: 51–58.

———, Branson, D. S., and Ameel, D. J. 1944 Effect of an all plant ration on the resistance of an omnivorous animal to parasitism. Tr. Kans. Acad. Sci. **47**: 215–218.

———, ———, ——— and Riedel, B. B. 1944 Substitution of soybean oil meal for animal protein in developing resistance of animals to parasitism. J. Parasit. **30**: (suppl.) : 12–13.

———, Fisher, M. L., and Zimmerman, N. B. 1927 Resistance to parasitism affected by the fat-soluble vitamin A. J. Parasit. **13**: 219–220.

——— and Nolf, L. O. 1931 Resistance of chickens to parasitism affected by vitamin B. Am. J. Hyg. **13**: 337–344.

——— and Spindler, L. A. 1929 Vitamin D and resistance of chickens to parasitism. Am. J. Hyg. **9**: 292–307.

Bamberger, J. W. and Martin, W. E. 1951 Effect of size of infective dose, partial ileectomy, and time on intensity of experimental infections of *Euhaplorchis californiensis* Martin, 1950 (Trematoda) in the cat. J. Parasit. **37**: 387–391.

Becker, E. R. 1933 Two observations on helminths. Tr. Am. Micr. Soc. **52**: 361–362.

Clapham, P. A. 1933 On the prophylactic action of vitamin A in helminthiasis. J. Helminth. **11**: 9–24.

———1934 Some observations on the effect of dietary deficiency on infestation of chickens with the nematode *Heterakis gallinae*. J. Helminth. **12**: 123–126.

Garcia, E. Y. 1936 Study on the resistance of the metacercariae of *Monorchotrema taihokui* to different physical chemical agents. J. Philip. Islands Med. Ass. **16**: 561–565.

Hager, A. 1941 Effects of dietary modifications of host rats on the tapeworm *Hymenolepis diminuta*. Iowa State College J. Sci. **15**: 127–153.

Levine, P. P. 1938 Observations on the biology of the poultry cestode *Davainea proglottina* in the intestine of the host. J. Parasit. **24**: 423–431.

Martin, W. E. 1950 *Euhaplorchis californiensis* n. g., n. sp., Heterophyidae, Trematoda, with notes on its life-cycle. Tr. Am. Micr. Soc. **69**: 194–209.

Reid, W. M. 1940 Some effects of short starvation periods upon the fowl cestode *Raillietina cesticillus* (Molin). J. Parasit. **26** (suppl.) : 16.

——— and Ackert 1941 Removal of chicken tapeworms by host starvation and some effects of such treatment on tapeworm metabolism. J. Parasit. **27** (6 Suppl.) : 35.

Riedel, B. B. 1946 Protein supplements and hydrogen-ion concentration as factors in the resistance of chickens to ascarid infection. Doctor's thesis. 77 pp. Kansas State College of Agriculture and Applied Science, Manhattan, Kansas.

——— and Ackert, J. E. 1950 The resistance of chickens to ascarids as affected by protein supplements of soybean oil meal and skim milk. Poultry Sci. **29**: 437–443.

Zimmerman, N. B., Vincent, L. B., and Ackert, J. E. 1926 Vitamin B, a factor in the resistance of chickens to *Ascaridia perspicillum* (Rud.). J. Parasit. **12**: 164.

THE TOXICITY OF SOME ALKYL SODIUM SULFATE DETERGENTS FOR HORSE STRONGYLE (NEMATODA) EGGS AND LARVAE

NORMAN D. LEVINE AND VIRGINIA IVENS

College of Veterinary Medicine and Agricultural Experiment Station
University of Illinois, Urbana, Illinois

Levine and Ivens (1953) reported that two detergents, Triton NE and Tergitol 7, were toxic for the eggs and larvae of horse strongyles. The former, a nonionic detergent, is a polyethylene glycol ether, while the latter is an anionic detergent whose active ingredient is an alkyl sodium sulfate. The high toxicity of the latter observed in this preliminary study made it desirable to investigate it and other related detergents more intensively. The present paper reports the effects of three alkyl sodium sulfate detergents on the eggs and larvae of horse strongyles. An abstract of a portion of this work has been published (Levine and Ivens, 1952).

MATERIALS AND METHODS

The three anionic detergents employed in this study were (A) the sodium sulfate derivative of 3,9-diethyltridecanol-6; this was used in a 25% aqueous solution which also contained 15% of the monobutyl ether of diethylene glycol as a coupling agent and 5% of a mixture of sodium sulfate and sodium chloride; (B) the sodium sulfate derivative of 7-ethyl-2-methylundecanol-4; this was used in a 25% aqueous solution which also contained 10% of the monoethyl ether of diethylene glycol as a coupling agent and 5% of a mixture of sodium sulfate and sodium chloride; and (C) the sodium sulfate derivative of 2-ethylhexanol; this was used in a 40% aqueous solution which also contained 5% of a mixture of sodium sulfate and sodium chloride. The trade names of the three detergents above (manufactured by the Carbide and Carbon Chemicals Co., New York) are respectively Tergitol 7, 4 and 08. They will be referred to in the text as A, B and C.

The effects of these detergents on horse strongyle eggs and larvae were studied in water and in horse feces. The strongyle eggs and larvae used in this study were obtained from the feces of horses belonging to the University of Illinois. Over 95% were those of small strongyles whose third stage larvae have eight intestinal cells.

The technics used in collecting, washing and treating the eggs and larvae in water have been described by Levine and Ivens (1953). Detergent A was tested against eggs, and against first, second and third stage larvae in concentrations of 0.1, 0.05, 0.025, 0.01, 0.005, and 0.0025%. Detergent B was tested against eggs at concentrations of 0.8, 0.6, 0.5, 0.1, 0.05, 0.025 and 0.01%; against first stage larvae in concentrations of 0.3, 0.2, 0.1, 0.05 and 0.025%; against second stage larvae at concentrations of 0.5, 0.3, 0.2, 0.1 and 0.05%; and against third stage larvae at concentrations of 0.8, 0.6, 0.5 and 0.1%. Detergent C was tested against eggs at concentrations of 5.0, 1.5, 1.0, 0.8, 0.5, 0.1 and 0.05%; against first stage larvae at concentrations of 1.0, 0.8 and 0.5%; against second stage larvae at concentrations of 1.5, 1.0, 0.8 and 0.5%; and against third stage larvae at concentrations of 5.0, 4.0, 3.0 and 1.0%.

Received for publication, October 20, 1952.

Three treated groups of eggs or larvae were set up at each concentration; two control groups were set up at the same time. Counts were made after 1, 2, 3 and 4 days of the numbers of eggs which had embryonated, the numbers of eggs which had hatched, and the numbers of larvae which were alive. The corresponding percentages were calculated and then corrected to take into account the percentages of the control eggs which had failed to embryonate or hatch or of control larvae which had died. These percentages were relatively small. After four days, 93–97% of the control eggs had embryonated, and 92–96% of them had hatched. After one day, 98–100% of the control first stage larvae and 99–100% of the control second and third stage larvae were still alive. After four days, 60–93% of the control first stage larvae, 87–91% of the control second stage larvae, and 96–100% of the control third stage larvae were still alive.

The average number of eggs used in each treated and control group was approximately 24. The average number of first stage larvae used in each treated group was approximately 22, and that in each control group approximately 21. The average number of second stage larvae used in each treated group was approximately 22, and that in each control group approximately 23. The average number of third stage larvae used in each treated group was approximately 21, and that in each control group approximately 22.

The *t* test was used to determine whether the observed differences between the treated and control groups were significant.

The technic used in testing the compounds against the developmental stages in feces has been described in detail by Levine (1950).

RESULTS

Washed eggs and larvae in water. For reasons of space, the complete results are not tabulated. The results can be summarized in two ways: either as the lowest concentrations at which the detergents had any effect at all, or as the lowest concentrations at which they killed all, or almost all, the larvae or eggs.

In terms of the former, and using the 1% level of significance as a criterion, 17% of the eggs were prevented from embryonating by 0.01% of Detergent A, 87% by 0.5% of Detergent B, and 100% by 1.5% of Detergent C.

Hatching of 58% of the eggs was prevented by 0.01% of the Detergent A, hatching of all eggs by 0.5% of Detergent B, and hatching of 68% of the eggs by 1.0% of Detergent C.

The first stage larvae were not affected by 0.005% of Detergent A in 2 days, but this concentration killed 91% of them in 4 days. They were not affected by 0.05% of Detergent B in one day, but 65% of them were killed by it in 4 days. They were not affected by 0.5% of Detergent C in one day, but 85% of them were killed in 4 days.

The second stage larvae were not affected by 0.005% of Detergent A in 2 days, but 36% of them were killed in 4 days. Forty-four percent of them were killed in 1 day and 99% in 4 days by 0.1% of Detergent B. They were not affected in 1 day by 0.5% of Detergent C, but 76% of them were killed in 4 days.

The third stage larvae were not affected by 0.025% of Detergent A in 1 day, but all of them were killed in 4 days. Eighty-eight percent of them were killed in 1 day by 0.05% of this detergent, and all of them were killed in 2 days. Thirty-six

TABLE 1.—*Lowest effective concentrations (%) of alkyl sodium sulfate detergents on horse strongyle eggs and larvae**

	Detergent A		Detergent B		Detergent C	
	1 Day	4 Days	1 Day	4 Days	1 Day	4 Days
Prevention of egg embryonation	0.05	0.05	0.6	0.6	1.5	1.5
Prevention of egg hatching	0.025	0.025	0.5	0.5	1.0****	1.5
Killing of 1st stage larvae	0.01	0.005**	0.2	0.1	0.8**	0.8
Killing of 2nd stage larvae	0.025	0.01***	0.2**	0.1***	1.5	0.8**
Killing of 3rd stage larvae	0.1**	0.025	0.6**	0.5**	4.0**	3.0

* Except where otherwise indicated, egg embryonation and hatching were completely prevented and all larvae were killed. Significant effects in which more than 10% of the eggs embryonated or hatched or in which more than 10% of the larvae were alive are not shown in this table.
** Less than 10% alive.
*** 1% alive.
**** 10% hatched.

percent of third stage larvae were killed in 1 day by 0.5% of Detergent B, and 97% of them in 3 days. Seventy-one percent of them were killed by 3.0% of Detergent C in 1 day, and all of them in 2 days.

In Table 1 are given the lowest effective concentrations of these detergents when 90% effectiveness is used as a criterion. In general, the first stage larvae were the least resistant, followed in order of increasing resistance by the second stage larvae, eggs and third stage larvae. Somewhat lower concentrations of the detergents prevented eggs from hatching than from embyonating.

Detergent A was the most toxic of the 3 detergents, and Detergent C the least toxic. For example, in killing first stage larvae, Detergent A was almost 20 times as active as Detergent B and almost 160 times as active as Detergent C. The relative activities of the three detergents against the eggs and second and third stage larvae were quite similar.

Eggs and larvae in feces. These detergents were much less effective in feces than in water. Detergent A and Detergent C killed or prevented the development of all larvae at a concentration of 2.5% but not at a concentration of 1.0%. Detergent B killed or prevented the development of all larvae at a concentration of 1.0% but not at a concentration of 0.5%. Thus, while Detergent A was the most effective of the 3 in clean water, Detergent B was the most effective in feces.

If one were to assume that these detergents acted primarily on the most sensitive stage, the first stage larvae, he could then estimate that Detergent A was 500 times less toxic in feces than in clean water, that Detergent B was 10 times less toxic in feces, and that Detergent C was approximately 3 times less toxic in feces. In this connection it might be mentioned that the manufacturer recommends the first detergent for use in solutions containing less than 1% acids, bases, salts, dyestuffs or

TABLE 2.—*Effect of 0.38% detergent A on horse strongyle eggs and larvae in feces*

	Days incub. at room temp.	Numbers of eggs or larvae per gram of feces			
		Unembryonated eggs	Embryonated eggs	Live larvae	Dead larvae
Treated feces	0	1500	0	0	0
	1	300	500	29	0
	2	71	57	700	0
	4	20	0	300	40
	8	0	0	100	164
Controls	0	1500	0	0	0
	1	300	1200	0	0
	2	20	40	1000	20
	4	14	0	900	100
	8	33	0	1300	0

starches, the second detergent for solutions containing 1–5% acids, bases or salts, and the third detergent for solutions containing more than 5% dissolved materials.

In order to get some idea of how these detergents act in feces, 4 experimental jars containing 0.38% of Detergent A in feces and 4 control jars were set up. At the time they were set up, the feces contained 1500 unembryonated eggs per gram. After 1, 2, 4 and 8 days, respectively, Stoll counts were made on one treated and one control jar.

The results are shown in Table 2. This concentration of the detergent is not sufficient to kill all the developmental stages, but it does kill the greater percentage of them. After 8 days, the original 1500 eggs were represented by 1300 live larvae in the control and 100 live larvae in the detergent-treated feces. There was no significant difference at any time in numbers of unembryonated eggs between the treated and control feces, or in numbers of embryonated eggs on any day except the first; after 1 day's incubation there were 500 embryonated eggs per gram in the treated feces and 1200 per gram in the control. At the same time there were 300 unembryonated eggs per gram in both jars. The peak of live larvae in the treated jar (700 per gram) was reached after 2 days' incubation, at which time there were 900 per gram in the control. Thereafter the number of live larvae in the treated feces dropped off, while that in the control remained about the same or increased. This is too small an experiment on which to base many quantitative conclusions, but it appears that the detergent acted on both the larvae and eggs. The swarm of bacteria in feces easily explains why relatively few dead larvae or eggs were observed.

DISCUSSION

Jaskoski (1950, 1951) has studied the effects of detergents on the eggs of the common swine roundworm, *Ascaris lumbricoides*. He found that 5.0% Duponol 80 (active ingredient, sodium octyl sulfate), Aresklene 400 (active ingredient, disodium dibutyl-*o*-phenylphenoldisulfonate), Areskap 100 (active ingredient, sodium butyl-*o*-phenylphenolsulfonate) and Aresket 375 (active ingredient, sodium butylbiphenylsulfonate) inhibited cleavage. In further studies, Jaskoski (1952) reported on the effects on *A. lumbricoides* eggs of Tergitol 08 (active ingredient, sodium octyl sulfate), Compound 1664 (active ingredient, sodium nonadecyl sulfate), Duponol 80, Product BC (active ingredient, *c*-betyl cetaine), and Sterox SK (active ingredient, polyoxyethylene ester). Of these detergents, Duponol 80 was by far the most effective in preventing cleavage. At 38° C., cleavage was prevented in normal eggs by 6 hours' exposure to 5% Duponol 80, and cleavage of "decoated" eggs (from which the protein outer shell layer had been removed by treament with antiformin) was prevented by 7 hours' exposure.

Jaskoski (1952) also studied the effects of these detergents on the hatching of the eggs after they had been allowed to incubate for at least 2 weeks prior to treatment. No normal eggs hatched when transferred to the detergent solutions, but hatching took place among the decoated eggs. The most effective detergent was 5% Duponol 80, in which all the eggs hatched in 3 hours at 38° C. In 5% Product BC, 80% of the eggs hatched in 8 hours. Hatching also took place in 100% Duponol 80 and Product BC. No eggs hatched in the other 3 detergents. Jaskoski does not mention the effects of the detergents on the hatched larvae. A comparison of Jaskoski's

findings with ours indicates that *A. lumbricoides* eggs are much more resistant to detergents than are horse strongyle eggs. It is of interest that in his work the detergents caused decoated *Ascaris* eggs to hatch, while in ours they prevented normal strongyle eggs from doing so.

SUMMARY

Three detergents whose surface-active ingredients are alkyl sodium sulfates were toxic for washed horse strongyle eggs and larvae in water. Embryonation of eggs was prevented by 0.05% of a detergent whose surface-active ingredient was the sodium sulfate derivative of 3,9-diethyltridecanol-6. In a concentration of 0.025%, this detergent prevented embryonated eggs from hatching; 0.01% killed all first stage larvae, 0.025% killed all second stage larvae, and 0.1% killed 94% of third stage larvae in less than a day. Embryonation of eggs was prevented by 0.6% of a detergent whose surface-active ingredient was the sodium sulfate derivative of 7-ethyl-2-methylundecanol-4, while 0.5% of this detergent prevented embryonated eggs from hatching, 0.2% killed all first stage larvae and 93% of second stage larvae, and 0.6% killed 95% of third stage larvae in less than a day. Embryonation of eggs was prevented by 1.5% of a detergent whose surface-active ingredient was the sodium sulfate of 2-ethylhexanol, while 1.0% prevented 90% of the embryonated eggs from hatching, 0.8% killed 94% of first stage larvae, 1.5% killed all second stage larvae, and 4.0% killed 94% of third stage larvae in less than a day. In general, somewhat lower concentrations were effective after 4 days' exposure.

In horse feces, it required 2.5% of the 3,9-diethyltridecanol-6 and 2-ethylhexanol detergents or 1.0% of the 7-ethyl-2-methylundecanol-4 detergent to kill all larvae or eggs.

LITERATURE CITED

JASKOSKI, B. J. 1950 The role of the protein coat in the development of the ova of *Ascaris lumbricoides* var. *suum*. J. Parasit. **36** (6 Sect. 2) : 33.

————1951 Effects of detergents on embryogeny of *Ascaris lumbricoides* var. *suum*. J. Parasit. **37** (5 Sect. 2) : 22.

————1952 The protein coat in development of *Ascaris lumbricoides* eggs. Exper. Parasit. **1**: 291–302.

LEVINE, N. D. 1950 The use of horse strongyle larvae in screening compounds for anthelmintic activity. Trans. Ill. State Acad. Sci. **43**: 233–236.

———— AND IVENS, V. 1952 The toxic action of some detergents on the eggs and larvae of horse strongyles (Nematoda: Strongylidae). J. Parasit. **38** (4 Sect. 2) : 32.

————, ————1953 The effects of some detergents on the developmental stages of horse strongyles (Nematoda). Trans. Ill. State Acad. Sci. **45**: 182–187.

MAINTENANCE OF *SCHISTOSOMATIUM DOUTHITTI* (CORT, 1914) IN THE LABORATORY (TREMATODA: SCHISTOSOMATIDAE)[1]

IRVING G. KAGAN,[2] ROBERT B. SHORT[3] AND MARTHA M. NEZ[3]

INTRODUCTION

In the United States, in addition to several avian schistosomes and *Heterobilharzia americana* found in the raccoon and lynx (Price, 1943), we have *Schistosomatium douthitti* occurring naturally in muskrats and field mice. *S. douthitti* is readily maintained in the laboratory and its life cycle is essentially the same as the species parasitizing man. In the present paper methods which have proved successful in the maintenance of *S. douthitti* in the laboratory and the results obtained are recorded.

Research with *S. douthitti* is adaptable to both the small and large laboratory. Since this parasite is not pathogenic for man (except for dermatitis) but produces typical pathology in laboratory animals it is excellent for teaching and demonstration purposes. Moreover, working with *S. douthitti* does not come under the jurisdiction of the federal quarantine laws and the precautions that must be employed with imported pathogenic material need not be observed.

The work herein reported was initiated in the laboratory of Dr. George R. La Rue at the University of Michigan. The data and observations are based on several years of independent research in Ann Arbor, Michigan (RBS), Chicago, Illinois and Philadelphia, Pennsylvania (IGK) and over one and one-half year's cooperative work in Tallahassee, Florida (RBS and MMN). Many of the methods outlined in the following paper were not developed by us. We have received much valuable advice and help from various workers interested in schistosomiasis. Visits to the laboratories of Dr. Eloise B. Cram and Mr. Nathan DeWitt, Laboratory of Tropical Diseases, National Institutes of Health, and Dr. Donald V. Moore, Department of Preventive Medicine, New York University Medical School have been most helpful.

HISTORICAL REVIEW

The first life cycle in the genus *Schistosomatium* was reported by Tanabe (1923a) for *S. pathlocopticum,* which he described as new using material recovered from white mice experimentally infected with cercariae from *Lymnaea palustris* (Müller), collected in or near Boston, Massachusetts. Price (1931) in Ann Arbor, Michigan, described the complete life history of *S. douthitti,* presenting detailed

Received for publication, October 30, 1953.

[1] The portion of this investigation done in Tallahassee, Fla. was supported by a research grant (E-346) from the National Microbiological Institute, of the National Institutes of Health, U. S. Public Health Service.

[2] The Zoological Laboratory, University of Pennsylvania, Philadelphia 4, Pa. A portion of this research was undertaken during the tenure of a National Research Council Postdoctoral Fellowship in the Department of Bacteriology and Parasitology, University of Chicago and was aided in part by Grant 844 of the Committee on the Advancement of Research of the University of Pennsylvania.

[3] The Zoology Department, Florida State University, Tallahassee, Fla. We wish to acknowledge the technical assistance of Mr. Roy Winslow.

morphological studies on the various stages. A comparative study by Penner (1942) of *S. pathlocopticum* and *S. douthitti* led him to synonymize *S. pathlocopticum* with *S. douthitti.*

The cercaria of *S. douthitti* was described by Cort (1914, 1915) from infected *Lymnaea reflexa* Say collected near Chicago, Illinois. Natural infections with *S. douthitti* have since been reported from the Douglas Lake region, Michigan in *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla* Walker by Cort (1917); in *Physa ancillaria parkeri* Currier by Cort (1918); in *Stagnicola exilis* (Lea), *S. palustris elodes* (Say) (= *Lymnaea palustris*), and *S. emarginata angulata* (Sowerby) by Cort (1936a). In addition, Brackett (1940) reported as snail hosts in Wisconsin: *Lymnaea stagnalis lillianae* F. C. Baker; *L. stagnalis jugularis* Say; and *L. stagnalis sanctaemariae* Walker; and Price (1931) reported *L. stagnalis appressa, L. palustris* (Müller); and *Physa gyrina elliptica* Lea as hosts near Ann Arbor, Michigan. If Penner's conclusion is accepted that *S. pathlocopticum* is a synonym of *S. douthitti,* Tanabe's (1923a) finding of *L. palustris* infected with this parasite in Boston, Mass., should also be added.

Studies on germinal development in the mother and daughter sporocysts of *S. douthitti* were made by Cort, Ameel and Olivier (1944). Recently their work on the daughter sporocysts was critically re-examined by Cort, Ameel and Van der Woude (1953). The results of these studies suggest that the method of multiplication of the intra-molluscan phase of *S. douthitti* fits the germinal lineage hypothesis with extended polyembryony.

The biology of *S. douthitti* cercariae was studied by Cort and Talbot (1936). It was shown that cercariae emerge soon after dark and after a brief free swimming period adhere to the surface film of the water by their acetabula. Olivier (1951) reported on the influence of light on the emergence of cercariae and was able to reverse the shedding cycle by placing snails in the dark during the day and under light at night. Dermatitis in man has been experimentally produced by *S. douthitti* cercariae (Cort, 1928, 1936a) and this species has been strongly suspected of causing some cases of dermatitis in persons wading in swampy areas (Cort, 1936b). Cort (1950) recently reviewed the literature on schistosome dermatitis and discussed the role of *S. douthitti,* among others, as a dermatitis producer. Infections in snails with single miracidia of *S. douthitti* were produced by Short (1952a) and the sex of the cercariae was determined. In agreement with results on other schistosomes, all cercariae from a single miracidium produced worms of one sex.

Natural infections of *S. douthitti* were found in two *Microtus p. pennsylvanicus* by Price (1931). She experimentally infected *Peromyscus maniculatus, Mus musculus,* rat and cat. She found that eggs were not passed in the feces of the rat and worms did not develop to complete sexual maturity in the cat. In addition to these hosts, Penner (1939) experimentally infected the muskrat, guinea pig, and snowshoe hare. His recovery of naturally infected muskrats from the vicinity of Minneapolis, Minnesota (32 of 330 examined) and the University of Michigan Biological Station (1 of 2 examined) led him to believe that young muskrats are the natural definitive hosts (Penner, 1938). Penner (1941) exposed a young rhesus monkey to thousands of cercariae and recovered schistosomulae in the lungs 5½ days later. Kagan (1953) recovered sexually mature worms in the portal system of rhesus monkeys necropsied 12–25 days after exposure. Infections in rhesus monkeys spontaneously terminated in this host 2–3 weeks after exposure.

The early morphological development of *S. douthitti* in the definitive host was studied by El-Gindy (1951), who gave special attention to the development of the sex organs. He reported that some worms may mature sexually as early as 10 days after infection. El-Gindy (1950) also investigated the route of migration from skin to portal system in mice, and contended that the young worms migrate from the lungs to the liver through the pleural cavity. Short (1952a) reported on the development of worms in mice with one and two sex infections. He found that both sexes mature regardless of the presence of the other, and maturity is attained in 12–13 days in both types of infection. Moreover, the eggs produced by female worms in unisexual infections develop parthenogenetically. Snail infections and resulting progeny of such uniparental miracidia were later reported (Short, 1952b). The same author also reported instances of female specimens with immature testes (1951). Short (1948), in further experiments with intergeneric crosses between *S. douthitti* and *S. mansoni,* found that female *S. mansoni* when with male *S. douthitti* developed to sexual maturity. Olivier (1952) studied the early pathology in mice exposed to *S. douthitti, S. mansoni* and *S. japonicum.* He found similarities in the pathology of the lungs caused by *S. douthitti* and *S. japonicum.*

Immunological studies have been made by a few investigators. Tanabe (1923b) studied the complement fixation test with *S. douthitti* and its group reaction with *S. japonicum.* Culbertson (1936) found that the cercariae of *S. douthitti* were resistant to the sera of many mammalian species but were killed by the sera of water snakes and green frogs. Kagan (1952) demonstrated that mice infected with *S. douthitti* develop an acquired immunity against superinfection. In a second study, Kagan and Lee (1953) found that approximately 2–3 weeks after effective chemotherapy, acquired immunity in mice was lost. Olivier (1949) demonstrated that schistosome dermatitis is a sensitization phenomenon and was able to sensitize human volunteers with cercariae of *S. douthitti* as well as with those of avian schistosomes. Immunological experiments with monkeys initially exposed to infections with *S. douthitti* and challenged with infections of *S. douthitti* and *S. mansoni* were made by Kagan (1953). He showed that monkeys were immune to the homologous species but not to the heterologous species. El-Gindy (1950) conducted experiments on immunity to reinfection in snails infected with *S. douthitti.* From his experiment he concluded that snails were immune to reinfection. Nolf and Cort (1933) reported that *L. stagnalis* infected with *S. douthitti* exhibited a partial, nonspecific immunity against the penetration and encystment of a strigeid cercaria.

Comparative chemotherapeutic studies of four antimonial compounds and Miracil D (1-diethylaminoethylamino-4-methylxanthone-hydrochloride) were made by Kagan and Lee (1952) with mice infected with *S. douthitti, S. mansoni* and *S. japonicum.* In this work, infections with *S. douthitti* were refractive to Miracil D, but responded to treatment with antimonials.

Porter (1938) reported *S. pathlocopticum* from an experimental infection in South Africa; but Penner (1942) pointed out the possibility that she was dealing with another species in the genus. A brevifurcate apharyngeal distome cercaria described as *Cercaria jaenschi* from *Ameria pyramida* and believed to be closely related to *S. douthitti* was described from Australia by Johnston and Cleland (1937).

SNAIL HOST

The snails used in our experiments are of two species, *Lymnaea stagnalis appressa* Say and *L. palustris* (Müller). Due to easier availability, *L. palustris* has been used more extensively and is being used exclusively at present. The original stock of *L. stagnalis* was obtained from Cheboygan Co., Michigan and of *L. palustris* from areas around Ann Arbor, Washtenaw Co., Michigan.

Snails infected with *S. douthitti* were collected for our work in several localities in both Michigan and Illinois. Near Ann Arbor, Michigan a collection of 665 *L. palustris* yielded 22 natural infections with *S. douthitti* (3%). Collections made by El-Gindy (1950) yielded 50 infections in 700 snails (7%). Near Chicago, Illinois, 50 infected snails were recovered in a collection of 600–800 *L. palustris* (6–8%) from small isolated ponds with large muskrat colonies. *S. douthitti* is probably endemic in many areas of the United States. Favorable places to seek infected snails are small pools or ditches near woods or isolated ponds with muskrat houses.

The methods of maintaining snails in our laboratories are similar to those used by other investigators (Noland and Carriker, 1946). Snails have been reared routinely in two types of containers: 1) glass aquaria, usually of 5 or 10 gallon capacity, and 2) shallow, white enameled pans (20 in. × 12 in. × 4 in.) with a capacity of 1½ gallons. A brief description of the methods employed follows.

Aquaria are started with 1 to 2 inches of thoroughly washed sand or fine gravel and aquarium plants. The presence of plants is not entirely necessary in an aquarium as is demonstrated by rearing snails for various periods without plants. The chief function of the plants seems to be furnishing places for attachment of egg masses and sometimes serving as a source of food. Water from the following sources proved satisfactory for rearing snail colonies: clear spring water; city tap water, which had been filtered through an activated charcoal filter to remove all traces of chlorine or allowed to stand in a large tank for several days or in carboys that were aerated; river water from the Wakulea River in Florida which is exceptionally clean and of constant chemical composition throughout the year.

Ordinary sticks of blackboard chalk or powdered calcium carbonate are supplied to the aquarium to provide material for the snails' shells. In addition, "vita-min" bricks, a commercial aquarium product, are added. These are said to contain calcium, phosphorus, iron, iodine, copper, zinc, manganese, magnesium and vitamins A, B, B_2, C, D, and G. They are recommended for aquaria with fishes, and apparently aid in the growth of snails, although controlled experiments in our laboratories have not been conducted to determine the extent of their value.

Food for the snails consisted of fresh, washed, green leaves of head lettuce, supplied every few days as needed. Rotting pieces of lettuce should be removed to avoid contamination of the water. Dried maple leaves are also excellent food; the leaves are soaked for a few weeks in several changes of water and then added to the aquaria. In addition, the water plants are eaten by the snails and must be replenished periodically.

The aquaria are aerated, either from compressed air outlets or by means of small electric aerators. Air is broken into fine bubbles in the aquaria by use of air stones attached to rubber or plastic tubing. Water in the aquaria is usually changed

every two to three months, at which time the sand is washed and the aquaria set up again as originally.

When pans are used, into each pan is put water, a small handful of gravel, dried maple leaves and a "vita-min" brick. In addition to the leaves, snails are fed lettuce or cabbage twice a week. The water in the pans is aerated and, unless the water becomes foul, snails do not climb out.

In addition to aquaria and pans, fingerbowls and small glass jars are sometimes used for maintaining snails in isolation for varying periods. The water in these containers is changed every day or two and fresh lettuce or dried maple leaves supplied as needed. Sometimes a small piece of chalk is also placed in each fingerbowl when the period of isolation lasts a week or longer.

The snails have been maintained at ordinary room temperatures, except in Tallahassee, Florida where they were reared in an air-conditioned room. The temperature in this room remained about 22–24° C., occasionally dropping to 21° C. or rising to 26° C. for short periods. A few snails have been reared at room temperature in Tallahassee and were able to withstand the prolonged hot summers of that area.

At temperatures of 22–24° C. stocks of *L. palustris* have grown well and produced egg masses practically the year round. Egg masses are usually attached to water plants or the dead maple leaves; sometimes the masses are also found on the sides of the aquaria. No special effort has been made to determine the minimum age at which egg-production begins under these conditions. It has been noted, however, that some infected snails deposited egg masses, with eggs which hatched, in isolation jars at 68–74 days of age (all snail ages computed from date of hatching).

Snails exposed to miracidia are usually young specimens. Young snails, of known ages, are obtained by removing egg masses from the stock aquaria to fingerbowls in about 150 cc. of water. Masses may be removed from the sides of aquaria by careful use of a razor blade. The water in these fingerbowls is changed every 2 or 3 days and records on time of hatching are kept. As commonly observed, hatching of all individuals in one egg mass does not occur simultaneously, but may extend over a period of 3 or 4 days. The young snails apparently derive some nourishment from the algae and bacterial growth on the plants and sides of the containers, and, in addition, tender lettuce leaves are added for food. The lettuce is apparently eaten more rapidly by these young snails if it is made tender by boiling about 1 or 2 minutes.

In stock aquaria, maintained chiefly as a source of egg masses, snails are removed occasionally to prevent excessive overcrowding; but generally no special effort is made to limit the number of snails. Snails exposed to miracidia are handdled more carefully. It has been found that 20–25 young exposed snails will live and grow well in a 5 gallon aquarium, while about twice that number do well in a 10 gallon tank. At the end of the incubation period, the snails are isolated and examined for cercarial production. Only the infected snails are returned to aquaria, leaving usually, fewer snails per aquarium than originally. Young progeny of the infected snails are removed periodically to avoid crowding and confusion with infected specimens.

Infected snails for which longevity records were kept were maintained for most

of their lives in relatively uncrowded conditions, with 2 to 13 snails per aquarium. Some of these snails have grown to a fairly large size, the largest of any in our laboratories. The largest of these specimens have shell lengths of 32 to 33 mm., sizes which compared favorably with those of the largest snails of this species in some natural populations.

Both infected and unifected snails have been successfully shipped hundreds of miles within the country and to France and Germany. Snails were placed in small perforated metal mailing containers surrounded by damp sphagnum and shipped air mail express. Large numbers of mature and young snails withstood periods of 5–7 days in transit and arrived alive.

The longevity of laboratory-reared snails is of some importance, especially when the snails are infected and it is necessary to have available a constant supply of cercariae for experimental purposes. Three snails with single miracidial infections lived for periods of 361, 635 and 687 days respectively after exposure (Short, 1952a). More recently additional data on longevity of infected snails have been

TABLE 1.—*Longevity of* Lymnaea palustris *exposed to 3–5 miracidia of* Schistosomatium douthitti

	No. snails	Age (days)	Duration of infection (days)
Exposed and infected		Minimum Ages	
4/16/52–4/27/52	61	29	...
Alive and shedding cercariae			
4/28/53	27	382	365
5/28/53	18	412	395
6/11/53	12	425	408
8/ 6/53	8	468	451
8/22/53	7	486	469
9/ 4/53	3	499	482
10/ 2/53	2	527	510
10/ 7/53	1	532	515
10/24/53	0	549	532
Exposed and infected			
9/25/52	12	149	...
Alive and shedding cercariae			
1/13/53	12	259	110
4/29/53	5	365	216
6/11/53	4	408	259
6/25/53	3	422	273
7/23/53	2	450	301
8/ 6/53	1	464	315
8/20/53	0	479	330

gathered from several lots of snails, exposed in small groups to several miracidia per snail. The data on 2 groups are presented in Table 1. One lot of 61 snails, 17–29 days old at time of exposure, were reared in the air-conditioned room (see above). The second group of snails were 149 days old at exposure and were maintained at room temperature. From the data in Table 1 it will be noted that under proper culture conditions, about half the infected snails may live to an age of 1 year; and that the duration of infection depends on the age of the snail when exposed. A few snails infected when young may carry infections for as long as 16–18 months. Of importance is the fact that snails up to 149 days, and probably older, can be readily infected. It is however usually desirable to expose young snails in order to obtain longer periods of cercarial production.

Miracidia for snail exposures may be obtained by hatching eggs present in host's feces or in liver tissue by using the side arm flask method described by McMullen and Beaver (1945) for bird schistosomes. This type of 1 liter flask is delineated

in Figure A. Our data do not indicate whether there is a difference in infectivity or viability of eggs hatched from feces or from the liver. Eggs obtained from both sources have given satisfactory results, but livers are usually used when large numbers of miracidia are desired. Water of the same types as used for rearing snails has proven satisfactory for hatching miracidia and exposing snails.

The methods used for obtaining miracidia from eggs in liver tissue follow the general techniques described by McMullen and Beaver (1945) for feces, with the following modifications: livers are torn into small pieces and then ground with a small amount of wet, coarse, clean sand, using a mortar and pestle or placed whole in a semi-micro Waring blender cup in a small amount of fluid and blended for 20 seconds. The ground or blended tissue may be directly transferred to the side arm flask with water or washed in several changes of 0.85% saline by decantation in a 500 cc. pharmaceutical graduate cylinder tapered at the bottom. Eggs of *S. douthitti* are inhibited from hatching by 0.85% saline. After the supernatant is clear, usually follow three 20 minute changes, the saline is carefully decanted and the material containing the eggs of *S. douthitti* transferred to the side arm flask with fresh water. After transfer is made the flask is filled first to the level of the dotted line (Fig. A), then to the brim, by rapidly pouring more water into the side arm. This procedure leaves clear water in the side arm. A paper bag with a hole of suitable size and position is then placed over the flask so that the arm protrudes, but the rest of the flask is covered. The flask is then placed 12–15 inches from a light source.

Miracidia, which are negatively geotropic and positively phototropic, swim into the clear water of the side arm, where they can be seen against a dark background with the naked eye. The first miracidia usually appear after about 5 to 10 minutes. Several hundreds or thousands of miracidia may hatch from a single liver and be swarming in the side arm within an hour after the flask has been filled and may be removed from the side arm with a pipette. After several hours, miracidia may cease to appear. Another group may sometimes be hatched by replacing most of the water in the flask. Water originally put in the flask is carefully siphoned off to a depth of about 1½ inches. A little water is added; the flask is vigorously shaken, then filled with water as originally. Placing the flask in a refrigerator overnight and replacing the water with water at room temperature the following morning will induce many new miracidia to hatch. This technique once yielded hundreds of miracidia per day over an 11 day period.

Snails have been exposed in groups to several miracidia per snail, individually to several miracidia, and to one miracidium each. Groups of snails were exposed in fingerbowls, stender dishes or occasionally aquaria. Snails were individually exposed most successfully to one or several miracidia in small stender dishes, vials or glass cups about 10 mm. diameter by 10 mm. deep. The small glass cups are handled more readily when several are cemented to small rectangular glass plates. Miracidia placed in these small cups may be observed under the binocular microscope and counted. After the miracidia and snails have been introduced, the containers should be covered to prevent escape of snails and evaporation of water. The snails are removed from the exposure containers after about 20–24 hours and reared in aquaria or pans until time for cercarial emergence.

Snails exposed to miracidia in the laboratory have been, with few exceptions, laboratory reared. In our work no special study has been made of variations to susceptibility of snails at different ages. Generally the snails exposed have been rather young, about 3 to 10 weeks old. However, the data of Table 1 show that young snails and considerably older snails were readily infected.

Data on the exposure of 1764 young snails of undetermined age reared in the laboratory in Michigan, Illinois and Pennsylvania over a 2 year period between March 1950 and December 1952 are presented in Table 2. Although these snails were of unknown age they were generally selected because of their small size and probably were 1 to 4 months old. Each snail was exposed individually in a small glass cup to several miracidia (generally not exceeding 10). The percentage of snails that became infected after exposure in the Florida series was significantly higher. Of interest is the fact that the snails used in the Florida work are the progeny of stocks sent from the Illinois laboratory. In the Florida series, snails ranging in age from 16 to 18 days to 149 days were exposed. There is no apparent significant variation in susceptibility of these different age groups. For example, in a group of 31 snails, 23–25 days old 19 were recovered after exposure and all

TABLE 2.—*Exposure of* Lymnaea palustris *and* L. stagnalis appressa *to several (less than 10) miracidia of* Schistosomatium douthitti

No. of snails exposed	No. of snails recovered	% survived	No. of snails infected	% snails infected[1]
1764	1252	71	293	23.4
381[2]	299	78	250	83.6

[1] Percentages calculated on the number of snails recovered.
[2] *L. palustris* maintained in air conditioned room in Tallahassee, Florida ; exposed to 3–5 miracidia each.

were infected. In another group of 15 snails 149 days old, 12 were recovered and all were infected. The rate of infection for all 15 lots ranged from 73.7% to 100%. Although all snails were exposed in the air conditioned room, 41 snails in 2 aquaria were maintained subsequently at room temperature. The percentages infected in these 2 tanks were 80% and 92.3%. It is evident, then, that the parasite developed as well in snails maintained at room temperature as in those reared in the air-conditioned laboratory.

Individual lots of snails exposed in Illinois yielded 50–80% infection. These snails were generally exposed during the spring of the year or early summer. During a winter period between December 18, 1950 and April 6, 1951, in Illinois, 380 snails in 16 lots averaged an infection rate of 5.5%. Although snails were exposed in warm rooms they were reared in rooms that were not well heated and the temperatures may have been below the minimum required for cercarial development.

The incubation periods were determined for 116 laboratory-reared *L. palustris,* infected with one miracidium each at 23–35 days of age and reared in an air-conditioned room. Snails were isolated daily before dark and examined the following day for cercariae. As can be seen in Figure B, incubation periods varied from 37–52 days after exposure, with the largest numbers of infections maturing at 42–44 days. Measurements of lengths of snail shells on day of first shedding average 14.2 mm., with a range of 5–23 mm. The mean number of cercariae shed on first day was 9.8 with a range of 1–55.

TABLE 3.—*Sex of cercariae obtained from snails, each exposed in the laboratory to several miracidia (less than 10) and snails naturally infected with* Schistosomatium douthitti

	No. of snails tested	Male-female infections	Female infections	Male infections
Laboratory infected snails	113	36 (31.8%)	26 (23.0%)	51 (45.1%)
Naturally infected snails	36	11 (30.5%)	10 (27.9%)	15 (41.7%)

The number of cercariae shed by an infected snail increases with time, especially during the first few days. Snails with infections over one year may produce several hundred cercariae in one evening's shedding. Snails in the laboratory do not generally produce as many cercariae as naturally infected snails. This fact may be the result of the smaller size usually attained by laboratory snails. It appears that the numbers of cercariae produced are probably positively correlated with the size of the snail when the daughter sporocysts are developing. Larger snails provide more space for development. We have also noticed what appears to be an increase in cercarial production over a period of several months. This increase appears to be positively correlated with growth of the snail after the end of the incubation period, suggesting that, even in mature infections, cercarial productivity increases as the snail grows. Some of the larger experimentally infected snails have yielded 500–1500 cercariae per day. As many as 1000–5000 cercariae per snail have been recovered from naturally infected *L. palustris* in one evening.

Observation of snails that were isolated daily for cercariae revealed that cercariae do not emerge every day. From some snails cercariae emerge for 2 to 3 days and skip a day or two. In one instance 7 infected snails yielding cercariae for several weeks ceased producing. Examination of the livers of these snails several weeks later revealed that the infections had terminated.

The sex of cercariae of 36 naturally infected snails was determined by infection of mice. In addition, 113 snails infected in the laboratory by exposures to several miracidia (Table 3) and 90 snails exposed to one miracidium each (Table 4) were tested. The percentage of infections in laboratory infected snails exposed to more than one miracidium and naturally infected snails was very similar. For snails exposed to a single miracidia the sex of cercariae infections did not deviate significantly from a 1 : 1 sex ratio.

TABLE 4.—*Infections in snails 15–150 days of age exposed to one miracidium of* Schistosomatium douthitti

Species	No. exposed	Age of snail in days	No. recovered and examined	Infected		No. for which sex of cercariae was determined	Male	Female
				No.	%			
L. stagnalis[1]	83	14–140	80	12	15.0	12	7	5
L. palustris[1]	121	14–150	110	12	10.9	12	7	5
L. palustris[2]	259	23–35	213	116	54.5	51	16	35
L. palustris	...		...	...	...	15	8	7
Totals	463		403	140		90	38	52

[1] Data from Short (1952a).
[2] Same snails as considered in Fig. B, for which incubation period was determined.

MAMMALIAN HOSTS

Mice, rats, hamsters, rabbits and monkeys have been infected with *S. douthitti* in our laboratories. The techniques for exposing these hosts varied with the purposes of the infection and were of two main types. Exposure by immersion in water containing cercariae was often used for routine maintenance of infected animals to provide eggs for obtaining miracidia. Several mice or hamsters were placed for a half hour in a battery jar or other small receptacle filled to a level of 1–1½ inches with water containing cercariae. Covered fingerbowls have been used for single mice and young hamsters and quart fruit jars proved excellent for large hamsters. Rabbits, after clipping the posterior part, have been exposed by immersion in a pail of water containing cercariae. Restraining the rabbit and immersing an ear in a finger bowl with cercariae is equally effective.

A second and more accurate method of exposure is to place cercariae directly on the host. The cercariae of *S. douthitti* after emerging from the snail cling to the surface film of the water and remain almost motionless. They can be lifted from the surface film of the water with a small loop, counted under a stereoscopic microscope and transferred to the skin of the host to be exposed. The loop is made from a strand of hair or the stylet from a 20 gauge hypodermic needle attached to the end of a wood applicator with household cement. In this manner, mice, hamsters, and monkeys have been exposed to known numbers of cercariae and quantitative data obtained.

Use of the loop technique requires that the mouse or hamster be taped to a small rectangular board (or piece of plastic 2½ inches × 6 inches for mice) or anaesthetized. The technique for taping mice down is essentially similar to that described by Olivier and Stirewalt (1952). With practice one operator can tape a mouse by holding the animal by the nape of the neck on its side against the board and passing the adhesive tape over the thorax and forelegs. The hind legs are either taped to the board or held in place with rubber bands attached to small nails fixed at the end of the board. The hair on the abdomen of the animal is clipped, the area is washed and moistened with water prior to placing the cercariae on the skin. Animals are returned to their cages ½ hour after exposure.

The use of an anaesthetic apparently does not interfere with infection. Intraperitoneal injection of sodium pentobarbital (Veterinary Nembutal, 60 mg. per cc.) has been used with success. For mice a 15% solution of pentobarbital in sterile distilled water administered intraperitoneally with a tuberculin syringe in the ratio of 1/100 cc. for each gram of body weight will anaesthetize for several hours. For hamsters a 20% solution administered in the same ratio is very effective. For rats, a 30% solution and administered in the ratio of 0.2 cc. per 100 grams body weight has been used. Monkeys have been anaesthetized with intraperitoneal injections of 1–2 cc. of undiluted pentobarbital.

Young worms begin to migrate from the liver to the mesenteric veins 10 or 11 days after infection (Short, 1952a); by the 13th day the majority are usually in the mesenteric veins, and after this age practically all worms (male–female infections) are found in the extra-hepatic portal vessels.

Sexual maturity is not attained at the same age by all worms, even in the same host. Some specimens of both sexes are usually mature at 10–12 days. Increasing numbers of worms become mature on subsequent days until the majority of both

sexes are mature by the 16th day and all (except anomalous ones) are mature at 20 days of age. The above data on sexual maturity were obtained from infections in deer mice (*Peromyscus maniculatus*) and albino mice (*Mus musculus*). Recent preliminary work using an inbred C3H strain of *Mus musculus* indicates the same pattern of development in this host.

Correlated with a difference in rate of maturity is a disparity in size and external differentiation of worms of the same age. Sexual dimorphism of some worms may be sufficiently evident at 10 days to separate sexes using a stereoscopic microscope (27 ×). Other worms of the same age or slightly older may be so undeveloped as to render sexing difficult or impossible by gross examination. By the 15th day the sex of practically all worms may be distinguished without difficulty. In deer mice, and probably in other hosts as well, maximum size of both sexes is reached at about 30–40 days.

Mature miracidia have been found in host tissues as early as 26 days after infection. We routinely obtain eggs with miracidia in feces and/or liver of hosts with infections of 40–150 days duration. The findings of Kagan and Lee (1952) suggest that miracidia of *S. douthitti* may live for only 3–4 weeks in host tissues. Animals with old infections do not produce as many hatchable eggs as hosts with infections of shorter duration.

Death of mammalian hosts is sometimes caused by infections with *S. douthitti* especially when excessive numbers of cercariae are used. Death often occurs about 3 days after exposure in very heavily infected mice (300–500 cercariae) or soon after 30 days in less heavily infected ones (100–300 cercariae). The former time coincides with the time of migration of young worms through the lungs, and death at this time apparently results from lung hemorrhages. After 30 days, death is apparently due to hemorrhage of the cecum and adjoining part of the large intestine caused by many eggs breaking through the intestinal wall. For mice, exposure to 50–75 cercariae of both sexes is recommended. For purposes of maintenance of the infection in the laboratory, hamsters of 50–80 gm. body weight should receive a similar dose, but large hamsters may receive up to 150 cercariae.

Data on longevity of adult worms demonstrate that their life span in favorable hosts can be well over one year. Female worms from unisexual infections survived for 468–484 days and both sexes lived in bisexual infections for 383 days (Short, 1952a). An infection with male worms was observed in a mouse infected for 828 days. These infections were in *Peromyscus* and were the oldest examined. Since the worms were in good condition, we may infer that they could have lived longer. Price (1931) reported a considerably shorter life span in the rat and Penner (1940) recovered a single pair *in copula* plus disintegrating worms from a rat infected for 236 days. In addition to a shorter life in the rat, our experience indicates that the worms in this host are usually smaller than those in mice. Further evidence of an immunological response of a cellular nature against infection with *S. douthitti* in the rat was the observation of several mature worms encapsulated in the lung of an infected rat. Rats, therefore, appear to be less favorable hosts for *S. douthitti* than mice; the worms in rats often dying off before the host, while in mice the life span of worms (once maturity is attained) is often, possibly in most cases, limited by the death of the host.

A number of rabbits have been exposed to the cercariae of *S. douthitti.* Infec-

tion in this host has been poor. Infections in monkeys persist for 2–3 weeks after which the worms are killed and destroyed. Specimens recovered from these animals prior to 3 weeks appear normal in all respects and after 13 days have reached sexual maturity (Kagan, 1953).

The hamster is now being used routinely in one of our laboratories for maintaining the mammalian phase of the life cycle. Livers of young hamsters exposed for over 30 days contain many eggs and make excellent preparations for obtaining miracidia with the side arm flask technique. Hamsters are very susceptible hosts. Infected animals are stunted in growth and show evidence of much pathology at necropsy.

Data on the susceptibility of various strains of mice exposed in our laboratories are given in Table 5. Mice exposed in Tallahassee, Florida, proved to be slightly more susceptible than the average for the series. Methods of infecting mice in this series differed in one respect from the described technique, in that the abdomen of the mouse was kept moist for the entire period of exposure with a fine spray of water. The mouse is very susceptible to infection. In approximately 1000 necropsies only one animal was found to be negative. The data in Table 5 are not considered

TABLE 5.—*Susceptibility of mice exposed by the loop technique to varying numbers of cercariae of* Schistosomatium douthitti

Mouse species	No. cercariae per mouse	No. mice	Total no. cercariae	Total no. worms	Average no. worms per mouse	% cercariae developing
CFl	10	9	90	41	4.55	45.55
CFl	25	93	2,325	829	8.91	35.65
CFl	50	313	15,650	7,644	24.42	48.84
C3H	50 & 60	30	1,630	942	31.36	57.79
White	60–80	11	745	438	39.81	58.79
CFl	100	14	1,400	767	54.78	54.78
dba	50 & 100	9	553	360	40.00	65.09
Totals		479	22,393	11,021	....	49.21

adequate for determining whether different inbred lines of *Mus musculus* vary in susceptibility to *S. douthitti.*

Infections of hosts may be standardized and very uniform worm counts obtained if an inbred line of mice are used and if cercariae are from a common pool of infected snails. Inbred lines of mice have yielded more uniform infections probably because of their genetic homogeneity. The studies of Evans and Stirewalt (1951) demonstrated the importance of using a common pool of cercariae of *S. mansoni* for purposes of infection to eliminate the element of variation in infectivity of cercariae.

As a general rule in our work snails were isolated late in the afternoon and cercariae used the same night several hours after emergence. Although studies on length of infective life of cercariae have not been made, cercariae used the following morning or early afternoon in the Florida series appear to be just as infective.

Worms may be recovered from experimental hosts by perfusing the systemic system with a solution of 0.85% sodium citrate and 0.85% saline. The technique described by Yolles, Moore, DeGiusti, Ripsom and Meleney (1947) is employed with slight modification. The mouse is etherized or killed by breaking the neck, and the skin is peeled back exposing the body wall. The body and pleural cavities are opened and the inferior vena cava between the liver and heart ligated with cotton thread and severed. The esophagus is cut and the lungs pushed aside exposing the

dorsal aorta. The carcass is then placed in a petri dish over a white enameled tray and the hepatic portal vein severed. A 20 or 22 gauge needle attached to the perfusing apparatus is inserted into the dorsal aorta and the systemic system perfused until the petri dish is almost full. The intact liver should swell considerably and blanch as the blood flows out. To insure that all worms have been washed out of the liver the needle should be inserted into the hepatic sinus and the liver further perfused. After perfusion both liver and mesenteries are removed and placed in separate petri dishes. The liver should be torn into small pieces with forceps in a small amount of saline to allow trapped worms to crawl out. A rapid method for recovery of residual worms within the liver is to place the intact organ in a semi-micro Waring blender cup, fill the well with saline and blend for 3 seconds. The liver is broken apart into small pieces and the worms released in good condition. The mesenteries are inspected under a stereo-binocular microscope or macroscopically with the aid of a binocular loupe against the white background of the enameled pan. Worms found in the mesenteric veins are removed from the blood vessels with fine curved sewing needles attached to small wooden handles. When data are desired on location of worms, the method as originally described by Yolles *et al* (1947) should be followed.

TABLE 6.—*Distribution of worm counts in 303 CF1 mice exposed to 50 cercariae each of* Schistosomatium douthitti

No. worms recovered	No. of mice
0– 5	8
6–10	18
11–15	20
16–20	43
21–25	52
26–30	62
31–35	57
36–40	29
41–45	8
46–50	6

In the absence of pressure for a perfusion apparatus, recovery of worms in mice is greatly facilitated by perfusing the animal using a 50 cc. syringe. Although this method does not remove all the worms, it dilutes the blood in the mesenteries with saline and facilitates finding and picking out the schistosomes.

The sex of 3642 worms recovered from 181 CF1 mice exposed to pooled cercariae of undetermined sex was tabulated. Male worms (55.35%) slightly outnumbered female worms. Slightly higher recovery of male worms in bisexual infections have been reported for other schistosome species, when pooled cercariae of unknown sex had been used.

The distribution of the number of worms recovered in 303 CF1 mice of both sexes exposed to 50 cercariae each is given in Table 6. In this entire lot all mice were infected; the lightest infections are represented by 6 mice, 2 each with 1, 2 and 3 worms; the heaviest infection by 1 mouse with 50 worms from 50 cercariae. The mean number of worms per mouse was 25.08 (50%).

DISCUSSION

The maintenance of *S. douthitti* in the laboratory does not present any particular problems that differ radically from the maintenance of other schistosome species. Rearing and infecting the snail host is perhaps the most difficult part of the life

cycle; however, with sufficient space and correct methods, lymnaeid snails may be reared in any quantity desired and readily infected.

FIG. A. Flask with side arm for the hatching and concentration of schistosome miracidia (from McMullen and Beaver, 1945).

In recent years there has been a considerable amount of work on the biology and maintenance of the schistosome species infecting man. Biologically *S. douthitti* has a life cycle that is similar to that of species infecting man. Our findings are in general accord with the results of other workers with *S. mansoni* or *S. japonicum.*

SUMMARY

For several years *Schistosomatium douthitti* has been maintained successfully in the laboratory. A description of the techniques used to rear snail colonies of *Lymnaea stagnalis appressa* Say and *L. palustris* (Müller) in aquaria, trays and fingerbowls is given. In addition are described: methods for obtaining miracidia from liver tissue, infecting young snails, obtaining cercariae from infected snails, exposing mice, hamsters, rabbits and monkeys to quantitative doses of cercariae, and a rapid method for the recovery of worms.

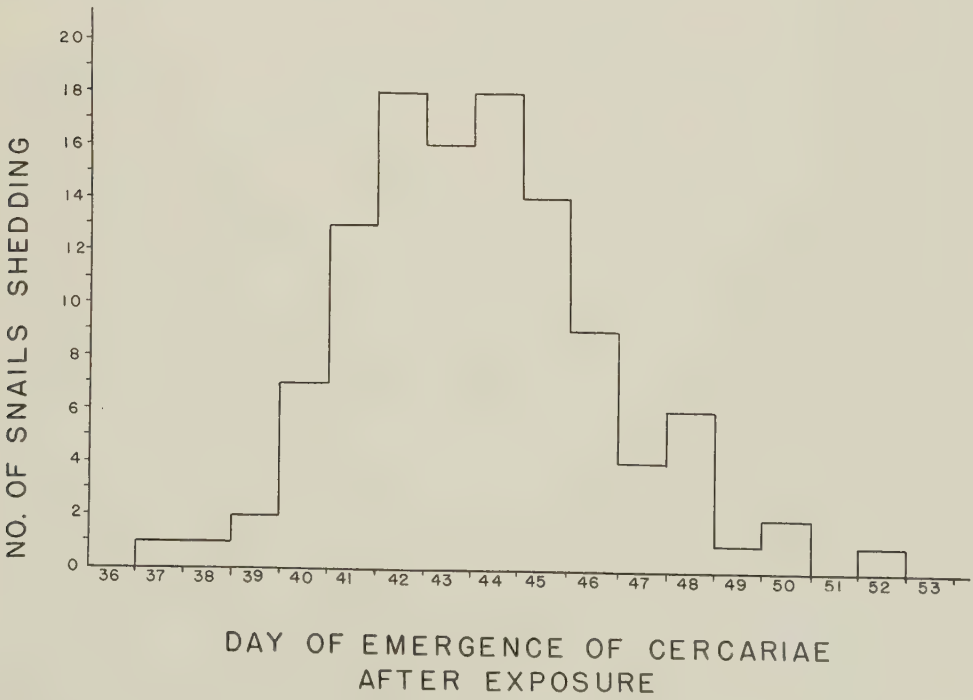


FIG. B. Lengths of incubation periods of *Schistosomatium douthitti* in *Lymnaea palustris.* Each snail was 23–25 days old when exposed to one miracidium.

Observations on the development of *S. douthitti* in the intermediate host are made. Data on the longevity of infected snails are given. The infectivity of laboratory reared snails to exposure of one and several miracidia is tabulated and the sex of cercariae for naturally infected snails, snails exposed to 5–10 miracidia and snails exposed to one miracidium each was determined by infection in mice. The incubation periods for snails infected with one miracidum each were determined.

Observation on the development of *S. douthitti* in the definitive host are made. The time required for worms to reach sexual maturity in *Peromyscus maniculatus* and albino mice was determined and data recorded on the longevity of infections. The susceptibility of various laboratory hosts are discussed and the number of worms recovered from a group of CF1 mice exposed to 50 cercariae each is tabulated.

References Cited

Brackett, S. 1940 Studies on schistosome dermatitis. VIII. Notes on the biology of the snail hosts of schistosome cercariae in Wisconsin and epidemiological evidence for the life cycles of some avian schistosomes. Am. J. Hyg. **32:** 85–104.

Cort, W. W. 1914 Larval trematodes from North American fresh-water snails. J. Parasit. **1:** 65–84.

———1915 Some North American larval trematodes. Ill. Biol. Monographs **1:** 447–532.

———1917 Homologies of the excretory system of the forked-tailed cercariae. A preliminary report. J. Parasit. **4:** 49–57.

———1918 Adaptability of schistosome larvae to new hosts. J. Parasit. **4:** 171–173.

———1928 Schistosome dermatitis in the United States (Michigan). J. Am. Med. Ass. **90:** 1027–1029.

———1936a Studies on Schistosome dermatitis I. Present status of the subject. Am. J. Hyg. **23:** 349–371.

———1936b Studies on schistosome dermatitis IV. Further information on distribution in Canada and the United States. Am. J. Hyg. **24:** 318–333.

———1950 Studies on schistosome dermatitis XI. Status of knowledge after more than twenty years. Am. J. Hyg. **52:** 251–307.

——— and Talbot, S. B. 1936 Studies on schistosome dermatitis III. Observations on the behavior of the dermatitis-producing schistosome cercariae. Am. J. Hyg. **23:** 385–396.

———, Ameel, D. J., and Olivier, L. 1944 An experimental study of the development of *Schistosomatium douthitti* (Cort, 1914) in its intermediate host. J. Parasit. **30:** 1–17.

———, ———, and van der Woude, A. 1953 Further studies on the early development of the daughter sporocysts of *Schistosomatium douthitti*. Proc. Helminth. Soc. Washington **20:** 43–49.

Culbertson, J. T. 1936 Cercaricidal action of normal serums. J. Parasit. **22:** 111–125.

Evans, A. S. and Stirewalt, M. A. 1951 Variations in infectivity of cercariae of *Schistosoma mansoni*. Exp. Parasitol. **1:** 19–33.

el-Gindy, M. S. 1950 Biology of *Schistosomatium douthitti* (Cort, 1914) Price, 1931 (Trematoda), in its hosts. Unpublished thesis, University of Michigan. (Available on microfilm, University Microfilms, Ann Arbor, Michigan, #2004).

———1951 Post-cercarial development of *Schistosomatium douthitti* (Cort, 1914) Price, 1931 in mice, with special reference to the genital system (Schistosomatidae-Trematoda). J. Morphol. **89:** 151–185.

Johnston, T. H. and Cleland, E. R. 1937 Larval trematodes from Australian terrestrial and freshwater molluscs. Part II. *Cercaria* (*Furcoceraria*) *jaenschi* n. sp. Tr. and Proc. Roy. Soc. South Australia **61:** 202–206.

Kagan, I. G. 1952 Acquired immunity in mice infected with *Schistosomatium douthitti*. J. Infect. Dis. **91:** 147–158.

———1953 Experimental infections of rhesus monkeys with *Schistosomatium douthitti* (Cort, 1914). J. Infect. Dis. **93:** 200–206.

——— and Lee, C. L. 1952 Chemotherapy of experimental infections of mice with *Schistosomatium douthitti, Schistosoma mansoni* and *Schistosoma japonicum*. J. Infect. Dis. **91:** 224–230.

——— and ———1953 Duration of acquired immunity of *Schistosomatium douthitti* infections in mice following treatment. J. Infect. Dis. **92:** 52–57.

McMullen, D. B. and Beaver, P. C. 1945 Studies on schistosome dermatitis IX. The life cycles of three dermatitis-producing schistosomes from birds and a discussion of the subfamily Bilharziellinae (Trematoda: Schistosomatidae). Am. J. Hyg. **42:** 128–154.

Noland, L. E. and Carriker, M. R. 1946 Observations on the biology of the snail *Lymnaea stagnalis appressa* during twenty generations in laboratory culture. Amer. Midland Naturalist **36:** 467–493.

Nolf, L. O. and Cort, W. W. 1933 On the immunity reactions of snails to the penetration of the cercariae of the strigeid trematode, *Cotylurus flabelliformis* (Faust). J. Parasit. **20:** 38–48.

Olivier, L. 1949 Schistosome dermatitis, a sensitization phenomenon. Am. J. Hyg. **49:** 290–302.

———1951 The influence of light on the emergence of *Schistosomatium douthitti* cercariae from their snail host. J. Parasit. **37:** 201–204.

———1952 A comparison of infections in mice with three species of schistosomes, *Schistosoma mansoni, Schistosoma japonicum* and *Schistosoma douthitti.* Am. J. Hyg. **55:** 22–35.

——— and Stirewalt, M. A. 1952 An efficient method for exposure of mice to cercariae of *Schistosoma mansoni.* J. Parasit. **38:** 19–23.

Penner, L. R. 1938 Schistosomatium from the muskrat, *Ondatra zibethica,* in Minnesota and Michigan. J. Parasit. **24:** (Suppl.) : 26.

———1939 Experimental studies on *Schistosomatium douthitti* (Cort) in mouse, rat, muskrat, guinea pig, and snow-shoe hare. J. Parasit. **25** (Suppl.) : 8–9.

———1940 Morphological and biological studies on certain helminths of the muskrat. Unpublished thesis, Univ. of Minnesota, submitted 1940.

———1941 The possibilities of systemic infection with dermatitis-producing schistosomes. Science **93:** 327–328.

———1942 Studies on dermatitis-producing schistosomes in eastern Massachusetts, with emphasis on the status of *Schistosomatium pathlocopticum* Tanabe, 1923. J. Parasit. **28:** 103–116.

Porter, A. 1938 The larval trematoda found in certain South African mollusca, with special reference to schistosomiasis (bilharziasis). Publ. South African Inst. Med. Res. **8:** 1–492.

Price, E. W. 1943 A redescription of *Heterobilharzia americana* Price (Trematoda: Schistosomatidae). Proc. Helminth. Soc. Washington **10:** 85–86.

Price, H. F. 1931 Life history of *Schistosomatium douthitti* (Cort). Am. J. Hyg. **13:** 685–727.

Short, R. B. 1948 Inter-generic crosses among schistosomes (Trematoda: Schistosomatidae). J. Parasit. **34** (Suppl.) : 30.

———1951 Hermaphroditic female *Schistosomatium douthitti* (Trematoda: Schistosomatidae). J. Parasit. **37:** 547–555.

———1952a Sex studies on *Schistosomatium douthitti* (Cort, 1914) Price, 1931 (Trematoda: Schistosomatidae). Am. Midland Naturalist **47:** 1–54.

———1952b Uniparental miracidia of *Schistosomatium douthitti* and their progeny (Trematoda: Schistosomatidae). Am. Midland Naturalist **48:** 55–68.

Tanabe, B. 1923a The life history of a new schistosome, *Schistosomatium pathlocopticum* Tanabe, found in experimentally infected mice. J. Parasit. **9:** 183–198.

———1923b Complement-fixation test of *Schistosomatium pathlocopticum* Tanabe, and its group reaction with *Schistosoma japonicum.* J. Parasit. **9:** 230–233.

Yolles, T. K., Moore, D. V., DeGiusti, D. L., Ripsom, C. A., and Meleney, H. E. 1947 A technique for the perfusion of laboratory animals for the recovery of schistosomes. J. Parasit. **33:** 419–426.

THE INTESTINAL HELMINTHS OF THE COYOTE *CANIS LATRANS* SAY, IN UTAH

JOSEPH MILES BUTLER AND ALBERT W. GRUNDMANN

Department of Invertebrate Zoology and Entomology
University of Utah, Salt Lake City

This study of the helminths of the coyote, *Canis latrans* Say, 1823, was initiated following the appearance in Northern Utah of 7 recent human cases of hydatid cyst infection produced by the larval form of *Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805. Carlquist and Dowell (1951) reported on 4 of these cases coming to them for surgical treatment during the interval of 1948 to 1950. Three additional cases have appeared subsequent to this, of which 2 were in children of pre-school age residing in Salt Lake City. Attempts to determine the definitive host of Echinococcus in this area led us to examine 200 dogs from Salt Lake City with negative findings (Butler and Grundmann, 1951) and to examine commercial silver fox, also with negative findings (Grundmann, Butler and Selin, 1953). The possibility that the coyote might act as the definitive host of this parasite and serve as a reservoir prompted the present study.

Cameron (1927), in a paper dealing with the human hydatid problem, reported the coyote as having been found naturally infected with *Echinococcus*. However, he failed to list the source of his information. Correspondence with workers in the field and a review of the rather extensive literature pertaining to the parasitic fauna of the wild CANIDAE of North America has failed to substantiate Cameron's report.

No previous study of the parasites of the coyote has been made in this region, and since this animal is an important predator, increased knowledge concerning its ecology is necessary to the understanding of the role it plays in the habitat, especially in regard to food habits.

MATERIALS AND METHODS

Our study is based upon helminths obtained from 75 preserved intestinal tracts (pylorus to anus) from freshly killed adult animals taken by hunters of the Predator and Rodent Control Division of the U. S. Fish and Wildlife Service in 13 counties of the State of Utah. The tract was tied off at each end at the time of removal and preserved in 10% formaldehyde. These gut specimens were labeled as to date and locality. In the laboratory, each tract was completely dissected beginning at the pyloric end. Dissections were performed in a black developing tray containing water. The intestine was looped back and forth 3 or 4 times by being attached to one end of the tray by means of dissecting hooks. As the intestine was opened a few inches at a time, it was rolled on a hemostat. All helminths found were removed and the entire mucosa examined with the aid of a 10 power hand lens. Four of each 10 inches of gut were removed and the mucosa examined under a stereobinocular microscope. Loose materials and feces were screened through a 100 mesh copper screen. Where formalin preserved material is used, this procedure yields better results than techniques commonly used with unpreserved material.

Received for publication, November 19, 1953.

Nematodes were cleared in lactophenol containing a small amount of concentrated picric acid to stain internal structures. Small nematodes and dissected portions of larger worms were mounted in clarite. The larger worms were stored in 70% alcohol and 10% glycerine. Each of the 1321 tapeworms recovered was stained with acid alum-carmine after being flattened between glass slides, cleared in oil of cloves and individually determined to species.

RESULTS

Dissection of the 75 intestinal tracts produced a total of 1576 helminths, 255 nematodes and 1321 tapeworms. Fifty-four animals, comprising seventy-two percent, were infected by helminths of 1 or more species. Round worms of 1 or more species were removed from 29 of the tracts, and cestodes of 1 or more species from 48. Twenty-nine coyotes possessed only 1 kind of parasite, 17 contained 2, 5 contained 3, and 3 animals contained representatives of 4 species.

Of the nematodes, *Toxascaris leonina* (Linstow, 1902) was predominant, occurring in 28 of the 75 coyotes examined, and comprised 233 of the 255 specimens recovered. *T. leonina* was first reported for the coyote by Stiles and Baker (1935). Sperry (1941) also found this species during his study of coyote food habits, but Erickson (1944) did not find a single specimen in the 65 coyotes examined by him. This species was also recovered from 7 of the 200 dogs from Salt Lake City examined by us. *T. leonina* is considered to be endemic in the wild carnivora of this region, and is also known to occur locally in the mountain lion, *Felix concolor hippolestes* Merriam. Ascarid infection is probably perpetuated in the vicinity of the dens where the soil is likely to become contaminated with eggs from infected adults and readily transferred to the young adults. Examination of 20 intestinal tracts from coyote pups, not included in the above 75, showed much higher infection than in adults and tends to confirm this observation. *Toxocara canis* Werner, 1782, the other common canine ascarid, was found but once in coyotes, although it appeared in 9 of the dogs from Salt Lake City.

Several species of Oxyuridae were recovered. Of these, *Dermatoxys veligera* (Rudolphi, 1819) has not hitherto been reported from the coyote. This nematode occurs commonly in the cecum of wild and domestic rabbits and is probably present in these animals throughout the United States. Our examination of wild cottontail rabbits of the genus Silvilagus in Utah also show this parasite to be quite prevalent and widely distributed in the State. A second species of Oxyurid, *Passalurus nonannulatus* Skinker, 1931 was also recovered. This species, also a parasite of wild rabbits, was described from *Lepus americanus* by Skinker (1931) who further reported it from the coyote *Canis lestes* (= *C. latrans lestes*). *P. nonannulatus* appears to be predominantly a parasite of the cecum of *Lepus californicus deserticola* Mearns, the jack rabbit, in this region. Considerable host specificity appears to be present in the case of these two roundworms with rare individuals of *Dermatoxys* being recovered from jack rabbits. Members of the two afore-mentioned species were recovered from the same intestinal tract and are considered as cases of spurious parasitism in the coyote.

Specimens of *Protospirura numidica* Seurat, 1914 were recovered in one instance during this study. Sperry (1941) in his study of the food habits of the coyote was the first to report *P. numidica* from coyotes collected in 6 western states. It is inter-

esting that this species appeared in the most heavily parasitized intestinal tract containing 217 helminths comprising 4 species. In addition to *P. numidica,* this coyote contained specimens of *Toxascaris leonina,* and of 2 tapeworms, *Mesocestoides kirbyi* and *Taenia pisiformis.*

Seven species of tapeworms were recovered during this study, the intermediate hosts of which reflect, to a degree, the food habits of the coyote in this region and the relative availability of these intermediate hosts as food. *Taenia pisiformis* Bloch, 1780 was the most prevalent cestode encountered both in the total number of worms recovered, and in the number and distribution of animals infected. A total of 54.6% of the tracts examined showed worms of this species. This may be contrasted with the study of dogs from Salt Lake City in which 1% of the dogs possessed worms of this species. The jack rabbit, *Lepus californicus deserticola,* has been found to be an intermediate host.

Three additional species of Taenia were recovered, viz. *T. rileyi* Loewen, 1929, *T. krabbei* Moniez, 1879 and *T. hydatigena* Pallas, 1766. *T. rileyi,* first reported for *C. latrans* by Erickson (1944), was recovered from 2 tracts. The intermediate host of this tapeworm is unknown, but is possibly a mouse. One coyote contained adults of *T. krabbei,* a species first reported from the coyote by Erickson (1944). Shaw, Simms and Muth (1934) recovered the cysticercus of this species from deer in Oregon and confirmed the determination by rearing 55 adults in the bobcat, *Lynx rufus.* Wardle and McLeod (1952) found the cysticercus in reindeer in Alaska. Another species common to CANIDAE, *T. hydatigena* was taken from 2 intestinal tracts. This species was first reported from *C. latrans* by Erickson (1944). The cysticerci of this tapeworm may be found in the abdominal cavities of sheep, cattle, goats, deer, pigs and squirrels throughout the United States.

Four coyotes contained *Multiceps packii* Christenson, 1929. Parasitism of the coyotes by this species was also first reported by Erickson (1944). The worm was described by Christenson (1929) who found the coenurus at the tip of the heart of the varying hare, *Lepus americanus phaenotus* Allen from Minnesota. The intermediate host in Utah is probably one of the rabbits.

Mesocestoides kirbyi Chandler, 1944 was recovered from 8 coyotes and may be considered to be the second most prevalent species in the coyotes of this region. Its life cycle has not yet been definitely determined although the *Tetrathyridium* (= *Cysticercus dithyridium* of Villot, 1883), commonly found in reptiles and birds has long been considered to be the larval stage. *M. kirbyi* was described from several lots of *Mesocestoides* collected from *C. latrans* in California by Chandler (1944). A new record for the coyote was obtained by the recovery of the common double-pored dog tapeworm, *Dipylidium caninum* (Linn., 1758) Leuckart, 1863 from a single coyote intestine.

The species of taenioid tapeworms present in the intestinal tract and the relative abundance of each can possibly be used as an indication of the proportion of the various prey species and carrion in the diet of the coyote. The 54.6 percent that possessed *Taenia pisiformis* demonstrates that rabbits make up a considerable portion of the diet and that they are widely parasitized. The 5.3 percent of infection of coyotes by *Multiceps packii* would indicate that these forms are less prevalent. Since the larval forms of *T. hydatigena* and *T. krabbei* are known to occur in cattle, sheep and wild ruminants such as deer, some food of this type must be taken by coyotes, probably in about the same proportion of diet as the percent of infection.

SUMMARY

The intestinal tracts of 75 adult coyotes, collected from 13 counties of Utah during the period of 1949 to 1952 were examined for helminths. The tracts were taken from freshly killed animals and preserved in the field in 10% formaldehyde.

Helminths of one or more species were recovered from 54 of the 75 animals. A total of 255 nematode specimens, comprising 5 species, were found. Of these, *Toxascaris leonina* Linstow was predominant in numbers. Others present were *Toxocara canis* Werner, *Protospirura numidica* Seurat, *Passalurus nonannulatus* Skinker and *Dermatoxys veligera* Rudolphi. The last named constitutes a new record for the coyote.

Seven species of tapeworms were found comprising 1321 specimens. *Taenia pisiformis* Bloch was most prevalent. Remaining forms were *Taenia rileyi* Loewen, *T. krabbei* Moniez, *T. hydatigena* Pallas, *Multiceps packii* Christenson, *Mesocestoides kirbyi* Chandler and *Dipylidium caninum* Linn. *D. caninum* is new to the coyote.

Although a search for the definitive host of *Echinococcus granulosus* (Batsch) prompted the study, this species was not recovered.

Literature Cited

Butler, J. M., and Grundmann, A. W. 1951 Fecal examination of 200 dogs from Salt Lake City, for intestinal helminths. J. Amer. Vet. Med. Assoc. **118:** 396–398.

Cameron, T. W. M. 1927 Some modern biological conceptions of hydatid. Proc. Royal Soc. Med. **20:** 272–283.

Carlquist, J. H., and Dowell, R. J. 1951 Echinococcus disease. Rocky Mt. Med. Jour. **48:** 773–776.

Chandler, A. C. 1944 A new species of *Mesocestoides, M. kirbyi,* from *Canis latrans.* J. Parasit. **30:** 273.

Christenson, R. O. 1929 A new cestode reared in the dog. J. Parasit. **16:** 49–53.

Erickson, A. B. 1944 Helminths of Minnesota Canidae in relation to food habits, and a host list and key to the species reported from North America. Am. Midland Naturalist **32:** 358–372.

Grundmann, A. W., Butler, J. M., and Selin, M. J. 1953 Examination of commercial silver fox as a possible host for *Echinococcus granulosus* (Batsch 1786) Rudolphi, 1805 in Salt Lake County, Utah. J. Amer. Vet. Med. Assoc. **123:** 235–236.

Shaw, J. N., Simms, B. T., and Muth, O. H. 1934 Some diseases of Oregon fish and game and identification of parts of game animals. Station Bull. (322) Oregon. Agric. Exp. Sta. pp. 1–23.

Skinker, M. S. 1931 Three new parasitic nematode worms. Proc. U.S. Nat. Mus. **79:** Art. 24, 1–9.

Sperry, C. C. 1941 Food habits of the coyote. Wildlife Res. Bull., U.S. Fish and Wildlife Serv. (4) 70 pp.

Stiles, C. W. and Baker, C. E. 1935 Key-catalogue of parasites reported for Carnivora (cats, dogs, bears, etc.) with their possible public health importance. Nat'l Inst. Health Bull. (163) pp. 913–1223.

Wardle, R. A. and McLeod, J. A. 1952 The zoology of tapeworms. Univ. of Minnesota Press, Minneapolis. 780 pp.

A NEW STRIGEID TREMATODE FROM THE PECTORAL SANDPIPER

Frank Fisher, Jr., and J. Dan Webster
Hanover College, Hanover, Indiana

On May 2, 1942, 700 trematodes were collected by the second author near Kemah, Texas, from the intestine of a single adult Pectoral Sandpiper, *Erolia melanotos*. There were no observable pathogenic effects due to the infection. Most of the trematodes were flattened, fixed in Bouin's fluid, stained with carmalum, cleared in cedar oil, and mounted in balsam, but 2 specimens were serially sectioned. Thirty-six whole mounts were studied thoroughly, although the measurements given below are based on only 10 whole mounts and the serial sections. Measurements in parentheses are averages from 10 specimens. All measurements are in millimeters.

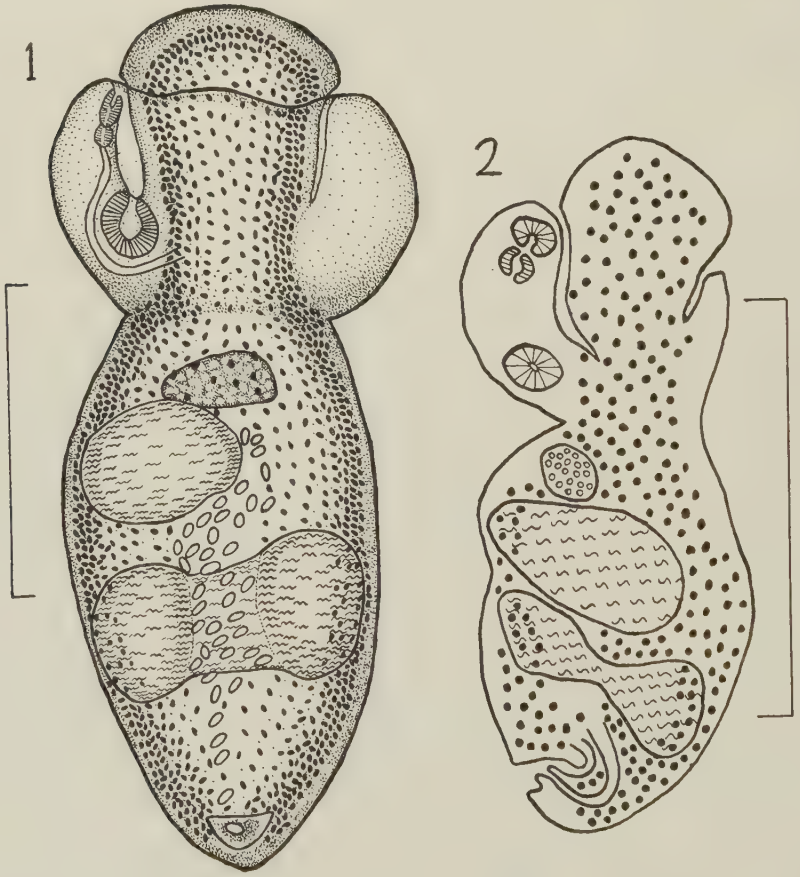


Fig. 1. Whole mount of *Strigea eroliae*, n. sp. Note the position and size of the holdfast organ. There is an egg in the genital atrium. As with most of our specimens, the worm is twisted so that the forebody is in lateral view, the hindbody in dorsal view. Scale 1 mm.

Fig. 2. Sagittal section of *S. eroliae* through anterior sucker, pharynx, and acetabulum reconstructed from three serial sections. Note the vitellaria in the extreme posterior end of the body. Scale 1 mm.

Received for publication, August 27, 1953.

Strigea eroliae, n. sp.

Diagnosis: Strigeidae: Forebody thick, cup-shaped, measuring 0.654 to 0.892 long and 0.714 to 0.999 wide (0.713×0.926). Holdfast organ thick, cup-like, protruding anteriorly from open end of forebody. Intestinal ceca twining through forebody and through hindbody almost to genital atrium. Hindbody broadly ovoid, ranging in size from 1.463×1.844 to 1.142×0.749 (1.315×0.957). Ratio of the length of the hindbody to that of the forebody 1.8: 1. Oral sucker spherical, 0.083 in diameter. Pharynx small, spherical, 0.071 in diameter. Ventral sucker distinguishable in four specimens, where it measures 0.154 to 0.178×0.107 to 0.154 (0.162×0.127).

Testes large, filling most of the hindbody, posterior testis the larger, ranging in size from 0.654×0.357 to 0.916×0.476 (0.776×0.448). Anterior testis measuring from 0.392×0.273 to 0.714×0416 (0.491×0.373). Posterior testis apparently bilobed; anterior ovoid or ellipsoid in all specimens examined. Ovary ellipsoidal, 0.130 to 0.178×0.249 to 0.321 (0.312×0.162) and located anterior to testes near union of two body segments. Vitellaria extending into extreme anterior part of holdfast organ, but not into the cup-like walls of the forebody itself, present throughout hindbody, being especially concentrated in its anterior portion. Eggs ellipsoidal, 0.047 to 0.059×0.071 (0.073×0.048), and present in all specimens. Genital atrium located very near posterior end of body, opening dorsally. Small bursa copulatrix within atrium and extending outward and anteriorly. Male genital pore located in end of bursa copulatrix. Vas deferens sphincter (*"Ringnapf"*) absent. Female genital pore anterior and ventral to bursa copulatrix.

Host: Erolia melanotos, the Pectoral Sandpiper.

Location: Small intestine.

Locality: Near Kemah, in Galveston County, Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 48789.

DISCUSSION

According to the key and general descriptions given by Dubois (1938), *Strigea intermedia* and *S. nicolli* resemble *S. eroliae* more than do other species of *Strigea.* However, *S. eroliae* differs from *S. intermedia* in the position of the holdfast organ and disposition of the vitellaria. In *S. eroliae,* the holdfast organ protrudes anteriorly and the vitelline follicles of the anterior region are confined to that organ, whereas in *S. intermedia,* the holdfast organ protrudes ventrally and vitelline follicles are found in the cupped walls of the anterior segment as well as in the holdfast organ.

S. nicolli differs from the present species in that the genital atrium is very large and is almost filled by the bursa copulatrix. *S. eroliae* has a small genital atrium and a very small bursa copulatrix.

Harwood (1931) described the North American variety *Strigea falconis meleagris* which resembles *Strigea eroliae* to some extent. However, *S. falconis* has a small holdfast organ, whereas *S. eroliae* has a much larger holdfast. Also, the genital atrium differs in its opening: that of *S. falconis* opens posteriorly and that of *S. eroliae* dorsally.

The present species is the first in the genus *Strigea* to be described from North American shore birds. For that matter, the genus *Strigea* has previously been recorded from shore birds of the suborder *Charadrii* only in Australia.

SUMMARY

Strigea eroliae, n. sp., is described from *Erolia melanotos* and means of distinguishing the species from three rather similar forms are given.

REFERENCES

DUBOIS, G. 1938 Monographie des Strigeida (Trematoda). Mém. Soc. neuchâtel. Sci. nat. **6**: 535.

HARWOOD, P. D. 1931 *Strigea falconis meleagris* new variety. J. Parasit. **18**: 51.

NOTES ON THE GENUS *MEGARTHROGLOSSUS* (SIPHONAPTERA, HYSTRICHOPSYLLIDAE) IN SANTA FE COUNTY, NEW MEXICO[1]

Harvey B. Morlan
P.O. Contract Station No. 1, Santa Fe, New Mexico

Incidental to a field ecology study of rodents and their ectoparasites by the Santa Fe Field Station, 32 specimens of *Megarthroglossus* have been collected from a variety of sources. The following observations are recorded to provide previously unpublished information on the comparative morphology of *Megarthroglossus bisetis* Jordan and Rothschild and *Megarthroglossus divisus divisus* (Baker) together with notes on the host relationships of these little known fleas.

Fleas of the genus *Megarthroglossus* are rarely collected. Hundreds of *Neotoma* and *Peromyscus* have been examined for ectoparasites by the Santa Fe Field Station, yet *Megarthroglossus* have been found only two times on *Neotoma* and one time each on three different species of *Peromyscus.* The few specimens of *Thomomys* and *Ochotona* examined have each provided one host record. Several specimens of *Tamiasciurus* have been examined without finding fleas of the above genus. *Megarthroglossus* have been taken from one of four nests of *Neotoma micropus canescens* and from six of 28 nests of *N. a. albigula.*

Megarthroglossus Bisetis Jordan and Rothschild

Megarthroglossus bisetis Jordan and Rothschild, 1915, Ectoparasites **1**: 54.
Megarthroglossus divisus bisetis Jordan and Rothschild. Hubbard, 1947, Fleas of Western North America, 833 pp., Iowa State College Press.
Megarthroglossus bisetis Jordan and Rothschild. Williams and Hoff 1951, U.S. Nat. Mus. Proc. **101**: 310–311. Description of the male.

The species was described from 2 females collected in August 1900 from *Neotoma* sp. at Beulah (San Miguel County), New Mexico as follows:

"Like *M. longispinus* [=*Megarthroglossus divisus divisus* (Baker)], but with only two antepygidial bristles on each side, the upper bristle being a little (one-fifth or one-sixth) longer than the lower one. The pronotal comb contains sixteen spines. The receptaculum seminis, which unfortunately is collapsed in both our specimens, is similar to that of *M. longispinus,* but the head is more regularly rounded, and the tail apparently somewhat shorter."

Collections from Santa Fe County, New Mexico contain 13 females that agree with the above description. In these specimens the number of pronotal spines was more often 15 but varied from 14 to 18. The posterior margin of sternum VII was irregular and usually without a sinus.

In describing the male of *M. bisetis,* Williams and Hoff failed to mention sternum VIII but stated that the pronotal comb is usually composed of 16 teeth; with a single antepygidial bristle on each side; and sternite IX broad at base, narrowed at apex. Their New Mexico records include one male from *Peromyscus maniculatus* collected near Bernalillo (Sandoval County) and many specimens from *N. m. canescens* or its nests collected near Albuquerque (Bernalillo County).

The 12 males collected in Santa Fe County agree in general with the description of Williams and Hoff. However, all of these specimens have three antepygidial

Received for publication, November 2, 1953.
[1] From the Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Ga.

setae on each side. In each case a long conspicuous seta is situated between two relatively minute setae. The number of pronotal spines was 14 on six specimens, 15 on two specimens, and 16 on four specimens. The posterior margin of sternum VIII is convex and the distal arm of sternum IX tapers to a narrowly rounded apex.

Megarthroglossus Divisus Divisus (Baker)

Pulex longispinus Baker 1895, Can. Ent. **27**: 131–132. Described from Georgetown, Colorado, *ex* "Fremont's chickaree" (*Tamiasciurus fremonti*).

Pulex divisus Baker 1898, Journ. N.Y. Ent. Soc. **6**: 54. New name for *P. longispinus* Baker 1895, which was preoccupied by *P. longispinus* Wagner, 1893.

Ceratophyllus divisus (Baker). Baker 1904, U.S. Nat. Mus. Proc. **27**: 388, 416, 441.

Megarthroglossus longispinus (Baker). Jordan and Rothschild 1915, Ectoparasites **1**: 52–53. An illustrated redescription.

Megarthroglossus divisus divisus (Baker). Hubbard 1947, Fleas of Western North America, 833 pp., Iowa State College Press. Lists records from a variety of hosts from California, Montana, Utah, and Alberta and states that the Rocky Mountain pine squirrels seem to be the favored host.

Megarthroglossus divisus divisus (Baker). Holland 1949, Canada Department of Agriculture Tech. Bul. **70**: 99. Lists records from British Columbia *ex Neotoma cinerea drummondi* and *Microtus longicaudus mordax*.

Santa Fe County, New Mexico records include five males and two females which are readily identified by the description and illustrations of either Jordan and Rothschild or Holland. Pronotal spines of the two females number 13 and 14; of the males, two have 12 spines, two have 13, and one has 14 spines. The males have one long antepygidial seta between two relatively minute setae.

SANTA FE COUNTY, NEW MEXICO RECORDS

M. d. divisus: 1 male and 1 female *ex Neotoma albigula albigula,* Oct. 26, 1951; 1 male and 1 female *ex* nest of *N. a. albigula,* Jan. 17, 1952; 1 male *ex* nest of *N. micropus canescens,* Mar. 25, 1952; 1 male *ex Peromyscus maniculatus rufinus,* May 6, 1953; and 1 male *ex Ochotona princeps incana,* Aug. 13, 1953.

M. bisetis: 1 female *ex Thomomys talpoides fossor,* Aug. 30, 1951; 1 male *ex Neotoma* sp., Sep. 6, 1951; 1 female *ex* nest of *N. a. albigula,* Oct. 10, 1951; 2 females *ex* nest of *N. a. albigula,* Nov. 29, 1951; 1 male *ex Peromyscus truei truei,* Jan. 11, 1952; 2 males and 2 females *ex* nest of *N. a. albigula,* Jan. 17, 1952; 1 female *ex* nest of *N. m. canescens,* Mar. 25, 1952; 1 male *ex* nest of *N. a. albigula,* Oct. 14, 1952; 5 males and 5 females *ex* nest of *N. a. albigula,* Nov. 18, 1952; 1 male and 1 female *ex* nest of *N. a. albigula,* Jan. 13, 1953; and 1 male *ex Peromyscus nasutus nasutus,* Jan. 22, 1953.

Most of the specimens of *Megarthroglossus* were collected from *Neotoma* nests taken at elevations between 6,300 and 6,900 feet above sea level. Rodents have been trapped at elevations of 5,500 to 11,000 feet. *M. bisetis* was collected at elevations from 5,500 to 9,000; *M. d. divisus* from 6,300 to 11,000 feet.

The few Santa Fe County records of *M. d. divisus* do not indicate any seasonal trend in abundance. Records of *M. bisetis* tend to show a winter peak of abundance and no collections have been made outside of the period from August 30 to March 25. It should be noted that on two occasions both species of fleas were found in a single *Neotoma* nest.

Males of *M. bisetis* and *M. d. divisus* are readily distinguished by the shape of sternum VIII and sternum IX; females by the number of antepygidial setae and

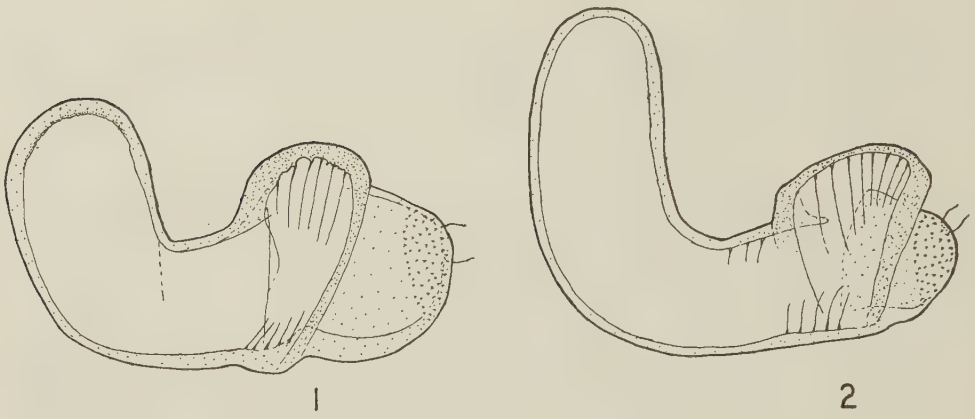


Fig. 1. Spermatheca of *Megarthroglossus bisetis.*
Fig. 2. Spermatheca of *Megarthroglossus divisus divisus.*

the shape of the spermatheca (receptaculum seminis). The posterior margin of sternum VIII of *M. bisetis* is convex; in *M. d. divisus* it is sinuate with a subventral lobe. In *M. bisetis* the distal arm of sternum IX tapers to a narrowly rounded apex; in *M. d. divisus* it is broadly rounded at the apex. There are two antepygidial setae on each side in females of *M. bisetis;* three in females of *M. d. divisus.* The spermatheca in *M. bisetis* (Fig. 1) has a relatively large head, narrow "collar," and short tail which may be slightly expanded subapically; in *M. d. divisus* (Fig. 2) the head is smaller, the "collar" broader, and there is no subapical expansion of the relatively longer tail.

GIGANTORHYNCHUS ORTIZI N. SP., AN ACANTHOCEPHALAN FROM *METACHIRUS NUDICAUDATUS*[1]

Luz Sarmiento

Department of Biology, Virginia Polytechnic Institute
Blacksburg, Virginia

In 1951, while on an expedition into the jungle in La Merced, Peru, Javier Ortiz de la Puente, ornithologist at the Museum of the University of San Marcos, Lima, Peru, collected a specimen of the rat-tailed marsupial, *Metachirus nudicaudatus.* The intestine of this animal contained 24 acanthocephalans which proved on further examination to belong to the genus *Gigantorhynchus.* Only 1 species, *G. echinodiscus* Diesing, was known in that genus until Machado (1941) described a second species, *G. lutzi,* from a Brazilian marsupial, *Caluromys philander.* The acanthocephalan here recorded is the first to be reported from *M. nudicaudatus* and is described as *Gigantorhynchus ortizi* n. sp. The specific name is in honor of the late Doctor Javier Ortiz.

Received for publication, August 19, 1953.

[1] A part of a thesis submitted to the graduate faculty of the Virginia Polytechnic Institute in partial fulfillment of the requirements for the Master's degree.

The writer wishes to acknowledge her indebtedness to Dr. W. L. Threlkeld for his encouragement, kindness, and helpful suggestions, and under whose supervision this work was carried out.

Dr. Machado very kindly sent a male specimen of *Gigantorhynchus lutzi* to the author for examination. Because of its condition, however, the specimen could not be readily compared with the parasite here reported.

MATERIALS AND METHODS

All specimens were fixed 8 days in 10 percent formalin, and then preserved in 70 percent alcohol. Different stains were used in order to show the complete morphology. The stains employed were picrocarmine, Mayer's carmalum, Reynold's stain, and Delafield's hematoxylin slightly acidified with acetic acid. Specimens were cleared in either xylene or wintergreen and mounted in permount. Two worms were prepared as serial sections stained with Delafield's hematoxylin-eosin.

The hooks were measured by means of a camera lucida. The following method was employed: the length of the hooks with double root from the base "C" to the tip following the median curvature of the hook, and the length of the root from the distal end "A" to the proximal end "B"; likewise, the hooks with simple root from the origin "D" to the tip including the root.

Gigantorhynchus ortizi n. sp.
(Figures 1–10)

Specific diagnosis: With the characters of the genus *Gigantorhynchus* Hamann, 1892. Body large and cylindrical, with imbricated pseudosegmentation, and divided into 3 portions by a dorsal and 2 lateral longitudinal grooves. A collar-like structure is present at 1.80 mm. posterior to the cuticular ring or neck fold. Body of females attenuated in both extremities measure 130 to 242 mm. in length and 1.50 to 2 mm. in maximum dorsoventral diameter. Males curved posteriorly measure 46 to 75 mm. long and from 1.4 to 1.92 mm. in maximum diameter at approximately the level of the cement glands.

The proboscis in both sexes is cylindrical and curved ventrally, neck short and devoid of hooks. Retractile praesoma is 1.45 to 1.72 mm. long and 0.435 to 0.555 mm. wide.

The proboscis is armed at the summit with 2 rows of 6 hooks each with double roots (Fig. 1). The dimensions of these hooks from their base "C" to the tip is approximately 0.160 mm. for the first row and 0.140 mm. for the second row. The length of the roots "A" to "B" is 0.100 mm. in the first row and 0.090 mm. in the second row (Fig. 3). The posterior portion of the proboscis is armed with small hooks with a simple root (Fig. 6), averaging about 0.050 mm. long. There are eleven or twelve of these hooks in each longitudinal row. According to the method of Van Cleave[2] (1941), the number of circles of hooks in this specimen is 21 or 23. The proximal circlets consist of numerous small hooks which increase in length as they approach the equatorial zone and decrease in length and number at the distal portion (Fig. 4).

[2] "In the writings of nineteenth century workers there is still confusion in the interpretation of their descriptions, whether they were recording the number of alternating circles of hooks or the number of hooks in each longitudinal row. The number of circles is twice the number of hooks in one longitudinal row, plus or minus one, for often adjacent vertical rows differ by one hook. Among present day morphologists it is very generally the practice to express the formula of proboscis hooks in number of longitudinal rows and number of hooks in each row, indicating the observed range of variability in both counts."

The proboscis receptacle is a closed muscular pouch suspended inside the anterior end of the proboscis. This pouch measures from 0.750 to 0.920 mm. long and does not receive the proboscis when it is inverted. In the specimen here described, the receptacle extends caudally half the length of the praesoma. Within the cavity are the invertor and retractor muscles, and between the invertor muscles lies a pyramidal mass of nerve cells which constitute the brain (Fig. 5).

The entire structure of the specimen, from the anterior tip to the cuticular ring, is here described as the praesoma, which includes the true proboscis and the neck (Van Cleave, 1950).

The lemnisci are long and cylindrical; they measure from 5.48 to 6.80 mm. in length by 0.129 to 0.163 mm. in width. The number of nuclei per lemniscus varies from 5 to 6. In the same specimen (Fig. 7) the number of nuclei observed in one lemniscus was 5, and 6 in the other.

A special arrangement of subcuticular nuclei of a fragmented type form the collar-like structure (Fig. 7) of the anterior trunk region. In addition to these nuclei, several other types of subcuticular nuclei have been observed throughout the body of the parasite. Immature specimens show spherical and ameboid types (Fig. 2), and older specimens display fragmented nuclei resembling those in the collar.

Males: The genital organs of the males are located in the posterior fifth of the body. The testes are elliptical and measure from 1.98 to 3.00 mm. long by 0.56 to 0.920 mm. wide. Just posterior to the testes are the 8 cement glands, closely grouped, but possessing distinctive walls and a single nucleus each (Fig. 10). The 2 vas efferentia unite within the cement glands to form a common sperm duct. The seminal vesicle and ducts of the cement glands are enclosed in the genital sheath. The penis projects into the bursa which is eversible.

Females: The ovary is represented by numerous small ovoid fragments, 0.150 to 0.270 mm. wide. The genital complex conforms with that usually described for this family. The vagina is short and muscular (Fig. 8). The eggs are elliptical and surrounded by 4 membranes, the exterior of which is smooth, but the shell within it is wrinkled. The eggs measure from 0.079 to 0.085 mm. in length by 0.049 to 0.054 mm. in width (Fig. 9).

Host: Metachirus nudicaudatus

Site: Intestine

Locality: Peru, South America

Type Specimen: U. S. Nat. Mus. Coll. No. 37433.

DISCUSSION

Of the two described species of *Gigantorhynchus, G. lutzi* is the only one which *G. ortizi* resembles enough to warrant further discussion.

The over-all length of *G. ortizi* exceeds that reported by Machado (1941) for *G. lutzi,* and the species differ in external appearance. *G. lutzi* is described as having 2 longitudinal grooves, a dorsal and a ventral, whereas in *G. ortizi* there are 1 dorsal and 2 lateral grooves. A further distinguishing feature of *G. ortizi* is the presence of a nuclear collar at the anterior portion of the trunk; such a collar is not mentioned for the other species of *Gigantorhynchus.*

The hooks at the summit of the proboscis in *G. ortizi* are smaller than in *G. lutzi,* whereas the hooks with simple roots are larger on the average. The arrangement

of small proboscis hooks also differs from that shown in Machado's drawing. In *G. ortizi,* the hooks are numerous and smaller in the first 6 or 7 circlets, and decrease in number at the equatorial zone. The number of these hooks in *G. lutzi* was not stated by Machado.

In Machado's figure of *G. lutzi,* the proboscis receptacle extends posteriorly as far as the neck fold, while in *G. ortizi* this pouch is much shorter and displays a cleft in the ventral side. In *G. lutzi,* the brain is at the posterior end of the pouch whereas in *G. ortizi,* the brain is not terminal. The lemnisci of these species differ both in relation to the length of the body and in respect to the number of their nuclei.

Marked differences in the genital organs of these species have also been found. In *G. ortizi* the testes are smaller, the cement glands are compact and not separated as in *G. lutzi,* and the ovarian fragments are smaller.

SUMMARY

A new species of Acanthocephala, *Gigantorhynchus ortizi* from *Metachirus nudicaudatus,* has been described and comparisons of its morphology have been made with the species of *Gigantorhynchus* (*G. echniodiscus* and *G. lutzi*).

References Cited

Machado, D. A. (*filho*) 1941 Sobre alguns acantocéfalos do estado do Pará. Rev. Brasil. Biol. **1**: 223–226.

Van Cleave, H. J. 1941 Hook patterns on the acanthocephalan proboscis. Quart. Rev. Biol. **16**: 157–172.

Explanation of Plate

All figures concern *G. ortizi* n. sp. Figure 4 was made with the aid of a projector and other figures with a camera lucida.

Fig. 1. Apical view of the proboscis showing the hooks with double root.

Fig. 2. Subcuticular ameboid nuclei.

Fig. 3. Hook with double root.

Fig. 4. Proboscis showing the arrangement of hooks.

Fig. 5. Praesoma showing proboscis receptacle and associated structures in detail.

Fig. 6. Hook with simple root.

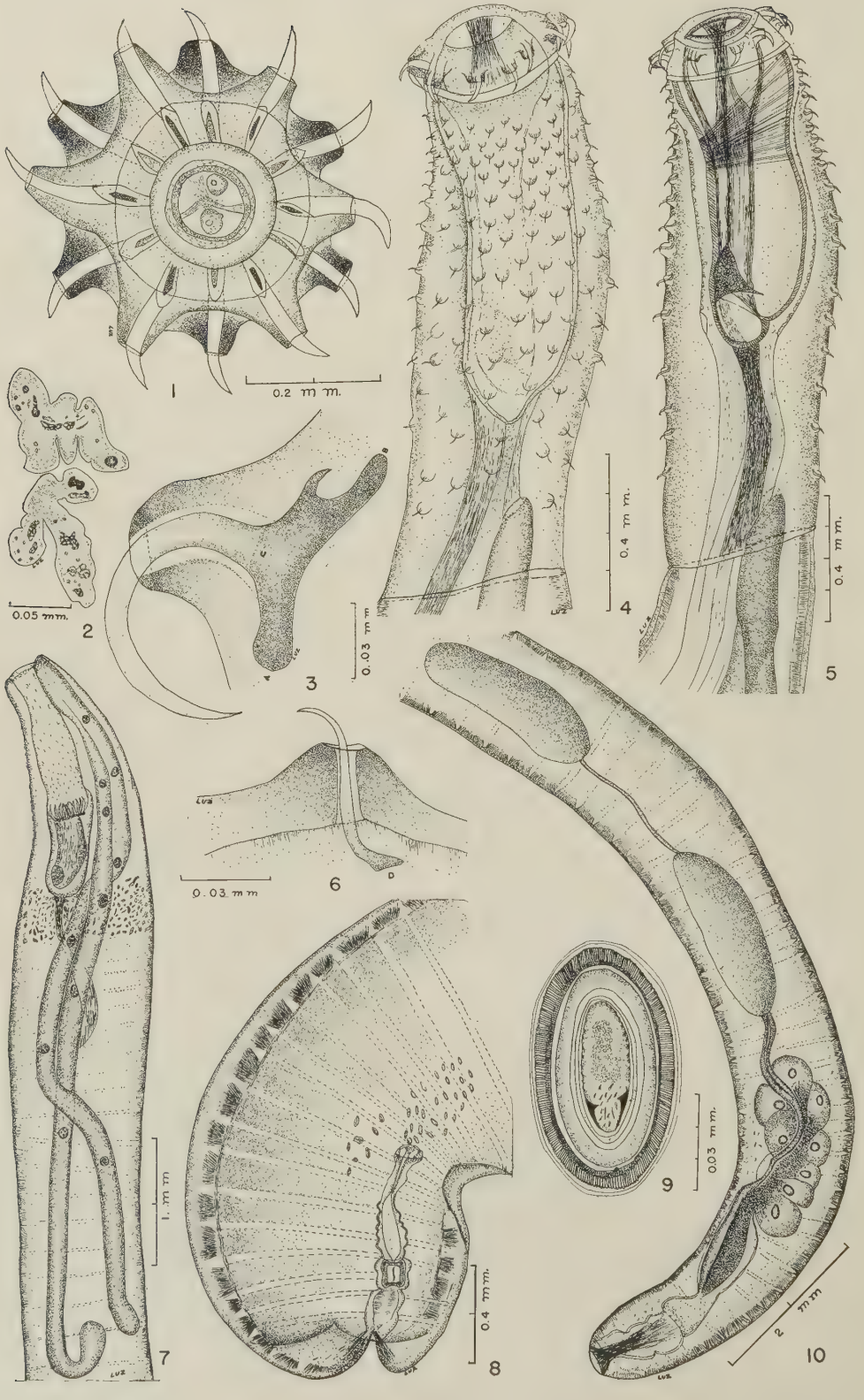
Fig. 7. Anterior portion showing inverted proboscis, lemnisci, and collar-like structure of nuclei.

Fig. 8. Posterior end of female.

Fig. 9. Egg showing smooth external membrane and wrinkled shell.

Fig. 10. Posterior end of male showing arrangement of genital organs.

PLATE I


0.2 m m.
1
0.05 mm.
2
0.03 mm
3
0.4 m m.
4
0.4 m m.
5
0.03 mm
6
1. m m
7
0.4 mm.
8
0.03 m m.
9
2 mm
10

SUSCEPTIBILITY OF SNAIL VECTORS TO GEOGRAPHIC STRAINS OF *SCHISTOSOMA JAPONICUM*[1]

WILLIAM B. DEWITT[2]

U. S. Department of Health, Education, and Welfare,
Public Health Service, National Institutes of Health,
National Microbiological Institute,[3] Bethesda, Maryland

In recent years several investigators have reported finding differences in the susceptibility of snail vectors to the schistosomes of man. Vogel (1941) reported that *Planorbis pfeifferi* (*Biomphalaria pfeifferi*) from West Africa was more susceptible to *Schistosoma mansoni* of West African origin than *Planorbis guadaloupensis* (*Australorbis glabratus*) from Dutch Guiana. Stunkard (1946) and Cram, Files, and Jones (1947) reported finding that *Planorbis boissyi* (*Biomphalaria boissyi*) from Egypt was refractory to *S. mansoni* from the West Indies. In addition, Files and Cram (1949) found that while *Australorbis glabratus* from Puerto Rico served adequately for the Puerto Rican strain of *S. mansoni, A. glabratus* from São Salvador, Brazil was completely refractory. Newton (1953) demonstrated that susceptibility to *S. mansoni* in *A. glabratus* is a heritable character.

In experiments with *S. haematobium* Cowper (1947) found that *Bulinus truncatus* of Egyptian origin had no attraction for miracidia of a strain from South Africa, and Barlow and Abdel-Azim (1948) reported that *Physopsis africana* from South Africa was susceptible to the Egyptian strain, whereas, *Bulinus tropicus* from South Africa was refractory.

The findings cited above, in addition to others reported, indicate that there are geographic strains of *S. mansoni* and *S. haematobium,* as well as certain snail hosts which, while morphologically identical with other strains, possess physiological differences affecting the host–parasite relationship.

The present paper reports a series of experiments, designed to yield information concerning the susceptibility of several snail hosts to *S. japonicum* from 3 distinct geographical areas.

While this work was in progress, Hunter, Ritchie and Otori (1952), working independently, found that wild specimens of *Oncomelania formosana* (Formosa) were unsuitable as hosts for *S. japonicum* autochthonous to Japan.

MATERIAL AND METHODS

Laboratory-reared specimens of 5 species of snails were employed in these experiments. The laboratory colonies were established from adult snails obtained as follows: *Oncomelania hupensis* from Shanghai, China, *O. nosophora* from Kofu, Japan, *O. formosana* from Chang Hua, Formosa, *O. quadrasi* from the islands of Leyte and Mindanao, in the Philippines, and *Pomatiopsis lapidaria* from along the banks of the Potomac River near Washington, D.C. *P. lapidaria* was included in this study because of its having been previously experimentally infected (Stunkard, 1946; Berry and Rue, 1948; and DeWitt, 1952) with *Schistosoma japonicum.*

Received for publication, November 23, 1953.

[1] Presented at the 27th Annual Meeting of the American Society of Parasitologists, September 10, 1952. Abstract published in *J. Parasit.* **38** (Supplement) : 35.

[2] With the technical assistance of Rodney H. Duvall.

[3] Laboratory of Tropical Diseases.

Schistosoma japonicum used in these experiments was obtained from infected snails included in the snail shipments from China, Japan, and Formosa, as listed above. The life cycle of each strain was established and maintained in the laboratory with the use of small mammals and snails from the laboratory breeding colonies.

Snail exposures were made with miracidia hatched from ova which were freed from infected liver tissue. Only freshly hatched miracidia were used. Snails were exposed either individually to 10 miracidia or in pairs to 15 miracidia. The exposures were made in small Syracuse dishes, allowing 12 to 14 hours for penetration. Tests were made in several series, using various numbers of snails; therefore the total number of snails exposed to any specific strain represents an aggregate of several experiments. An attempt was made to include a cross section of snails ranging in size from juveniles to fully matured specimens.

After exposure, the snails were kept in clay saucers or glass bowls which contained moistened dirt, leaves and twigs. From time to time small amounts of tropical fish food were added. Sixty days after exposure the snails were isolated in small tubes of water for observation. Those snails found to be negative for cercaria were returned to the saucers and held for 5 subsequent examinations at weekly intervals. At the end of this observation period the snails still remaining negative were examined by dissection; those found to contain cercariae were considered as positive.

RESULTS

A total of 303 *O. hupensis,* 313 *O. nosophora,* 499 *O. quadrasi,* 644 *O. formosana,* and 1208 *P. lapidaria* were exposed to *S. japonicum* from China, Japan, and Formosa. The results of the exposures, which are shown in Table 1, reveal that

TABLE 1.—*Results following exposure of* Oncomelania hupensis, O. nosophora, O. formosana, O. quadrasi, *and* Pomatiopsis lapidaria *to* Schistosoma japonicum *from China, Japan, and Formosa*

Source of *S. japonicum*	*Oncomelania hupensis* from China				*Oncomelania nosophora* from Japan				*Oncomelania quadrasi* from Philippines				*Oncomelania formosana* from Formosa				*Pomatiopsis lapidaria* from U.S.A.			
	E	L	+	%	E	L	+	%	E	L	+	%	E	L	+	%	E	L	+	%
Shanghai, China	64	53	18	34	85	68	0	0	114	98	0	0	154	150	0	0	480	345	4	1
Kofu, Japan	131	113	15	13	105	87	18	21	105	91	0	0	136	123	0	0	564	499	0	0
Chang Hua, Formosa	108	97	0	0	123	67	14	21	280	245	1	0.4	354	315	111	35	164	107	3	3

E = Total number of snails exposed.
L = Total number of snails alive at end of observation period; 90–100 days.
+ = Number of snails positive for *S. japonicum.*
% = Percentage of snails positive as based on the total number of snails alive at the end of the observation period.

O. hupensis (China) became infected with *S. japonicum* from China and Japan, but was refractory to *S. japonicum* from Formosa. *O. nosophora* (Japan) became infected with Japanese and Formosan strains but could not be infected with the Chinese strain. *O. quadrasi* (Philippines) was completely resistant to the development of the Chinese and Japanese strain, and only a very small percentage of the snails exposed to the Formosan strain became infected. *O. formosana* (Formosa) was readily infected with its native Formosan strain, but was refractory to the other two strains. Small numbers of *Pomatiopsis lapidaria* became infected with both Chinese and Formosan strains, but no evidence of infection was found when they were exposed to the Japanese strain.

DISCUSSION

The experiments reported in this paper contribute information on the compatibility of parasite and snail host in the life cycle of *Schistosoma japonicum.* Snail vectors from some endemic areas were found to be capable of serving as the intermediate host of *S. japonicum* from more than one geographical area, while others were not so broadly adapted. This is diagrammatically shown in figure 1. In addition evidence was obtained that suggests possible basic physiologic differences between *S. japonicum* of different endemic areas. For example, it was found that *S. japonicum* from Shanghai, China could infect *O. hupensis* from the same area, but not *O. nosophora* from Kofu, Japan, while *S. japonicum* from Kofu, Japan could infect snails from either area, and *S. japonicum* from Chang Hua, Formosa could infect *O. nosophora* from Kofu, Japan but not *O. hupensis* from Shanghai,

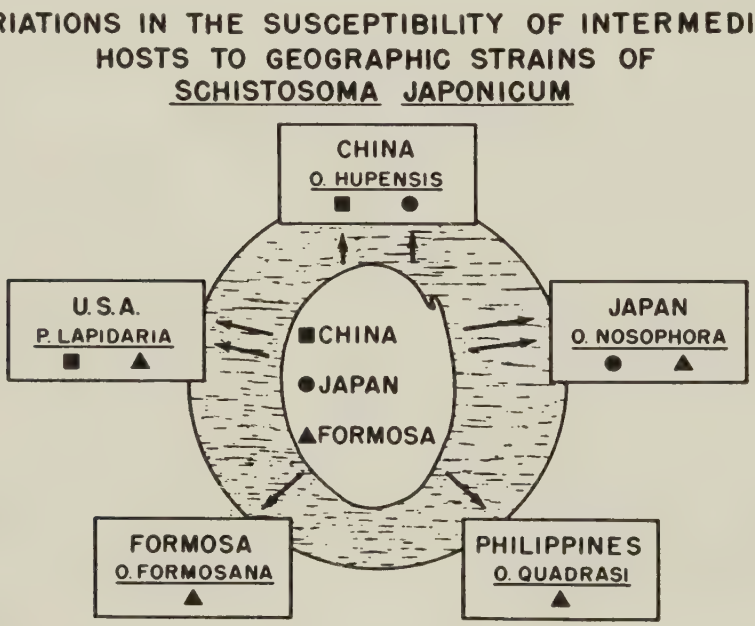


FIG. 1. Variations in the susceptibility of intermediate hosts to geographic strains of *Schistosoma japonicum.*

China. From this, it seems reasonable to conclude that the three strains of parasites are physiologically different.

It can be noted from the table that 5 species of snails were exposed to 3 strains of *S. japonicum,* resulting in 15 different snail-parasite combinations. Of the 15 groups, 8 were found to have some degree of compatibility. Among these 8 groups, 3 were found to have infection rates of 3 per cent or less while the others ranged from 13 to 35 per cent. This would seem to suggest that compatibility between snail and parasite is one of degree, and that the degree of compatibility is dependent on physiologic characteristics of both host and parasite.

SUMMARY

In this study, laboratory-reared *Oncomelania hupensis* originating from Shanghai, China; *O. nosophora* from Kofu, Japan; *O. formosana* from Chang Hua, For-

mosa; *O. quadrasi* from Leyte and Mindanao, P.I.; and *Pomatiopsis lapidaria* from the Potomac River near Washington, D.C. were exposed to strains of *Schistosoma japonicum* from Shanghai, China; Kofu, Japan; and Chang Hua, Formosa. *O. hupensis* was readily infected with the Chinese and Japanese strains but in these tests could not be infected with the Formosan strain. *O. nosophora* was susceptible to both the Japanese and Formosan strains but was refractory to the Chinese strain. *O. formosana* became infected with the Formosan strain but could not be infected with the Chinese and Japanese strains. *O. quadrasi* was susceptible only to the Formosan strain while *P. lapidaria* was infected with the Chinese and Formosan strains but not the Japanese strain.

References

Barlow, C. H. and Abdel-Azim, M. 1948 5th Annual Report of Bilharzia Snail Destruction Section 1946–1947. Ministry of Public Health, Cairo, 37 pp.

Berry, E. G. and Rue, R. E. 1948 *Pomatiopsis lapidaria* (Say), an American intermediate host for *Schistosoma japonicum*. J. Parasit. **34** (6 Suppl.): 15.

Cram, E. B., Files, V. S., and Jones, M. F. 1947 Experimental molluscan infection with *Schistosoma mansoni* and *Schistosoma haematobium*. Bull. (189) Nat. Inst. Health pp. 81–94.

Cowper, S. G. 1947 Observations on the life-cycle of *Schistosoma mansoni* in the laboratory, with a discussion on the snail vectors of *S. mansoni* and *S. haematobium*. Ann. Trop. Med. and Parasit. **41**: 173–177.

DeWitt, W. B. 1952 *Pomatiopsis lapidaria*, its occurrence in the Washington, D.C. area and its laboratory rearing in comparison to that of *Oncomelania* spp. J. Parasit. **38**: 321–326.

Files, V. S. and Cram, E. B. 1949 A study on the comparative susceptibility of snail vectors to strains of *Schistosoma mansoni*. J. Parasit. **35**: 555–560.

Hunter, G. W., III, Ritchie, L. S., and Otori, Y. 1952 A comparison of the infectivity of *Schistosoma japonicum* occurring in Japan for *Oncomelania nosophora* and *Oncomelania formosana*. J. Parasit. **38**: 492.

Newton, W. L. 1953 The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus*. Exper. Parasit. **2**: 242–257.

Stunkard, H. W. 1946 Possible snail hosts of human schistosomes in the United States. J. Parasit. **32**: 539–552.

Vogel, H. 1941 Infektionsversuche an verschiedenen Bilharzia-Zwischenwirten mit einem einzelnen Mirazidium von *Bilharzia mansoni* und *B. japonicum*. Zentralbl. Bakt. I Abt. Orig. **148**: 29–35.

EXPERIMENTAL INFECTION OF THE AFRICAN RELAPSING FEVER TICK, *ORNITHODOROS MOUBATA* (MURRAY), WITH *BORRELIA LATYCHEVI* (SOFIEV)

Willy Burgdorfer[1] and Gordon E. Davis[2]

The Asiatic relapsing fever spirochete, *Borrelia latychevi*, was isolated by Sofiev (1941) in Middle Asia from rodents, *Rhombomys opimus* and *Gerbillus eversmanni*, and from its natural vector, the argasid tick, *Ornithodoros tartakovskyi*. Recently, Baltazard and collaborators (1952) reported the unsuccessful attempts to transmit this spirochete by *O. erraticus* (small and large form) and *O. normandi*

Received for publication, December 18, 1953.

[1] Medical Entomologist, Research Fellow, Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana.

[2] Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

and stated that *O. tholozani* and *O. moubata,* as well as the North American argasid ticks, could not serve as vectors of this organism.

In studies of the life cycle of spirochetes in various tick vectors, the development and persistence of *B. latychevi* in *O. moubata* were observed. It is the purpose of this paper to report these observations which are partially in disagreement with those of Baltazard *et al.*

MATERIALS AND METHODS

The *O. moubata* used for the following experiments were from clean stock maintained at the Rocky Mountain Laboratory. This stock originated from ticks collected in the Belgian Congo by Dr. R. Geigy, Director of the Swiss Tropical Institute. Dr. M. Baltazard, Director of the Pasteur Institute, Teheran, Iran, kindly sent us infected *O. tartakovskyi* from which a strain of *B. latychevi* was isolated. This species of relapsing fever spirochete produces only a mild infection in adult rabbits, white mice, and man and fails to infect white rats, gunea pigs, or dogs (Sofiev, 1941; Pospelowa-Shtrom, 1940). New-born rabbits, however, are highly susceptible, since from 70 to 100 per cent of these animals, infected either by tick bite or by inoculation of infectious blood, die in 15 to 20 days. During this period, a steady increase in the number of spirochetes in the peripheral blood can be observed (Baltazard *et al.,* 1952).

In order to carry out the experiments to be described, large numbers of ticks and infected hosts were needed. The following procedure was employed. Infected *O. tartakovskyi* were allowed to feed on a normal white mouse. Tail blood of this animal was examined daily. As soon as spirochetes were observed, the blood was used to inoculate groups of normal mice. When these animals were known to have spirochetemia, second and fourth nymphal stages and adults of *O. moubata* were allowed to feed on them. After the ticks had engorged, they were stored at room temperature in desiccator jars in which a relative humidity of 79 per cent was maintained by a saturated solution of ammonium chloride. In order to follow the behavior of *B. latychevi* in *O. moubata,* several ticks were dissected each day and their organs were examined by dark-field technique according to methods described by Burgdorfer (1951).

In addition to this procedure, haemolymph and coxal fluid from the ticks were examined for spirochetes. The haemolymph was obtained by amputating the distal portion of the legs, the coxal fluid by applying a warm needle or forceps to the ventral side of the ticks, which respond to such irritation with the expulsion of this fluid.

Smears prepared from the blood of infected mice were examined under the microscope, using a magnification of 450 and ordinary direct illumination.

RESULTS

1. *Infection and persistence of* B. latychevi *in second nymphal stage* O. moubata. Two hundred *O. moubata* in the second nymphal stage were fed on a mouse which had in the peripheral blood an average of two to three spirochetes per microscopic field. Fifty-three of 90 ticks examined during the first 75 days after the infective feeding were shown to be carriers of active spirochetes. The organisms were regularly found in the haemolymph, central ganglion, coxal organs, and salivary glands after the fifth day following the infective feeding. An increasingly heavy infiltra-

tion could be observed in the central ganglion and in the coxal organs, whereas in the salivary glands fewer spirochetes were found as the period of infection increased.

2. *Infection and persistence of* B. latychevi *in fourth nymphal stage* O. moubata. One hundred and ten *O. moubata* in the fourth nymphal stage were fed upon mice which had in the peripheral blood an average of four spirochetes per microscopic field. Twenty days after the ticks had engorged, the survival of *B. latychevi* was checked by examination of haemolymph. Spirochetes in varying numbers were found in 70 of 110 ticks studied. All of the ticks which did not show spirochetes in the haemolymph were dissected and examined. Heavy infections were noted in central ganglions, coxal organs, and salivary glands of 15 of these 40 ticks. Spirochetes could also be found in the walls but never in the lumen of the Malpighian tubules. Direct examinations of ticks previously shown to be infected revealed that *B. latychevi* persists in the tissues for more than 150 days.

3. *Infection and persistence of* B. latychevi *in adult* O. moubata. Fifty adult *O. moubata,* both males and females, were fed on infected mice whose peripheral blood contained an average of three spirochetes per microscopic field. Thirteen of the ticks were dissected and tested at intervals from 10 to 340 days after the infective

TABLE 1.—*Transovarial transmission. Presence of* B. latychevi *in eggs and progeny of infected* O. moubata *as revealed by dark-field examination*

Tick No.	Date of Infection	Date of Oviposition	No. of Eggs			No. of Nymphs		
			Tested	Positive	Negative	Tested	Positive	Negative
1	7–7–52	8–13–52	33	16	17	None		
2	"	9–15–52	50	0	50	25	0	25
3	12–6–52	4–5–53	30	30	0	20	12	8
4	"	"	15	12	3	20	12	8
5	"	4–6–53	15	15	0	25	17	8
6	"	"	15	14	1	15	1	14
7	"	6–14–53	21	15	6	None		
8	"	"	26	18	8	20	15	5

feeding. It was found that *B. latychevi* developed and persisted also in adult *O. moubata.* The spirochetes penetrate the gut wall, enter the body fluid, and then attack the various organs. Central ganglion, coxal organs, salivary glands, and the walls of the Malpighian tubules are regularly parasitized. In addition, the spirochetes were found in the tissues of the genital system of adult female ticks, especially in the ovarian tissue and egg follicles. The genital system of male ticks was not examined. Examination of eggs from 8 and of nymphs from 6 infected female ticks indicated that transovarial transmission can occur. Spirochetes could be found in eggs of 7 and in nymphs of 5 infected *O. moubata* (see Table 1).

4. *Transmission experiments.* Theoretically, *O. moubata* could transmit relapsing fever spirochetes by (a) excretions of the Malpighian tubules, (b) coxal fluid, which is ejected shortly before the tick finishes feeding, and (c) saliva, *i.e.,* by bite. Although the spirochetes were found in each of the organs which secrete these fluids, transmission through the excretions of the Malpighian tubules would not be expected since no spirochetes were found in the lumen of this organ or in the so-called "tick feces."

The coxal fluids of several hundred *O. moubata* were examined for the presence of spirochetes. With a few exceptions all fluids contained spirochetes in varying numbers. Seventy-three transmission experiments were then performed to determine whether or not infected *O. moubata* could transmit *B. latychevi* to adult white

TABLE 2.—*Number of spirochetes in peripheral blood of adult mice, suckling mice, and new-born rabbits inoculated with* B. latychevi

	Number of spirochetes* found																	
	Days after inoculation																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Adult mice																		
No. 1	0	0	1	45	3	4	1	4	14	8	1	–	1	1	0	0	1	0
No. 2	0	0	1	59	4	1	1	3	4	0	0	–	0	0	0	0	0	0
Suckling mice																		
No. 1	0	1	1	15	92	7	43	52	47	4	48	–	15	11	5	14	4	8
No. 2	0	1	2	27	79	32	39	39	140	4	12	–	14	12	0	1	1	8
New-born rabbits																		
No. 1	0	0	20	202	300	300	104	130	21	108	164	+						
No. 2	0	1	16	180	140	300	300	80	120	+								

– = Not examined.
+ = Died.
* The number of spirochetes was estimated by counting the organisms observed over a period of 5 minutes in a blood smear stained by Giemsa's method.

mice or new-born rabbits. Ticks in lots of 3 to 10 were employed in each experiment. None of the animals became infected.

To explain the negative results, it was postulated that either the quantity of spirochetes transmitted by saliva and coxal fluid was not sufficient to produce a frank infection in the animals or the virulence of *B. latychevi* had been decreased by passage through *O. moubata.* In order to determine which of the above explanations was valid, a comparative study was made of the course of infection in experimental animals inoculated either with suspensions of infected *O. moubata* or with blood from a mouse upon which infected *O. tartakovskyi* had fed.

Six suckling mice, 6 adult mice, and 6 new-born rabbits were inoculated with 0.25 ml. of citrated blood (12 drops of tail blood containing one spirochete per microscopic field, diluted in 2.0 ml. citrate solution) from a mouse which had been infected by *O. tartakovskyi.* All animals became infected. The course of infection in the peripheral blood of two animals of each group is shown in Table 2. It was found that the number of spirochetes in the blood of adult mice was less than the number in the blood of suckling mice and that spirochetemia persisted for a greater length of time in the latter animals. In new-born rabbits, however, an extremely heavy infection occurred, which caused death of all the animals.

In a parallel experiment, the animals were inoculated with suspensions of infected *O. moubata.* The ticks had been fed on infected mice and were then held for periods of 90 to 355 days before they were used. Varying numbers of ticks were triturated in 1.0 to 3.0 ml. of saline, and 0.25 ml. of the suspension, which was proved to contain spirochetes, was inoculated intramuscularly into suckling mice,

TABLE 3.—*Results of attempts to infect mice and rabbits by injecting suspensions of* O. moubata *infected with* B. latychevi

No. of triturated ticks	Days after infective feeding	Suckling mice		Adult mice		New-born rabbits	
		Positive	Negative	Positive	Negative	Positive	Negative
10	92	0	6	–	–	–	–
10	92	–	–	1	5	–	–
2	104	–	–	0	6	0	2
1	138	–	–	0	4	–	–
2	184	–	–	–	–	1	1
1	210	–	–	3	4	–	–
4	212	–	–	–	–	0	4
3	212	–	–	–	–	1	3
1	355	–	–	–	–	1	3

– = None injected.

adult mice, and new-born rabbits. As will be noted in Table 3, only a few animals became infected and in most instances the inocula did not produce infection.

DISCUSSION

Since *B. latychevi* persisted and multiplied in the tissues of *O. moubata,* including the coxal organs and salivary glands, there was a possibility that this species of tick would transmit these spirochetes as did *O. tartakovskyi.* However, none of the mice or new-born rabbits used in transmission experiments became infected, and a reason for the apparent discrepancy was sought. *B. latychevi* is relatively less virulent than other species of relapsing fever spirochetes, but it does cause a frank infection in mice and particularly in new-born rabbits when transmitted by its natural vector, *O. tartakovskyi,* or when infected blood is employed as an inoculum (see Table 2). The fact that suspensions of heavily infected *O. moubata* inoculated into these animals failed, with a few exceptions, to produce an infection suggests that *B. latychevi,* by passage through *O. moubata,* partially loses its original virulence.

SUMMARY

B. latychevi persists through all stages of *O. moubata* studied. It is found consistently in the central ganglion, the coxal organs, the salivary glands, the walls of the Malpighian tubules, and in the tissues of the genital system, as well as in the progeny of infected ticks.

Attempts to infect mice and new-born rabbits by feeding infected *O. moubata* were unsuccessful, and inoculation of suspensions of heavily infected ticks into these animals failed, except in a few instances, to produce infection. The results of these experiments indicate, therefore, that there is a change in the virulence of *B. latychevi* induced by passage through *O. moubata.*

REFERENCES

BALTAZARD, M., BAHMANYAR, M., POURNAKY, R., AND MOFIDI, CH. 1952 *Ornithodorus tartakovskyi* Olenev 1931 et *Borrelia* (*Spirochaeta*) *latychevi* Sofiev 1941. Ann. Parasit. **27**: 311–328.

BURGDORFER, W. 1951 Analyse des Infektionsverlaufes bei *Ornithodorus moubata* (Murray) und der natürlichen Uebertragung von *Spirochaeta duttoni.* Acta Trop. **8**: 193–262.

POSPELOWA-SHTROM, M. V. 1940 *Ornithodoros tartakovskyi* Ol. 1931 as a vector of tick spirochaetosis. Med. Parasit. & Parasitic Dis. Moscow, **9**: 618–622. [In Russian.]

SOFIEV, M. S. 1941 *Spirochaeta latychewi* n. sp. of the *recurrens* group. Med. Parasit. & Parasitic Dis. Moscow, **10**: 267–271. [In Russian.]

THE EFFECTS OF VARIOUS ENVIRONMENTAL FACTORS ON THE HATCHING OF EGGS OF *PLAGITURA SALAMANDRA* HOLL (TREMATODA: PLAGIORCHIIDAE)[1]

CATHERINE M. RUSSELL
University of Virginia, School of Medicine
Charlottesville, Virginia

Plagitura salamandra Holl, 1928 is an intestinal parasite of the spotted newt, *Triturus v. viridescens* (Rafinesque) and uses the snail *Pseudosuccinea columella* (Say) as its intermediate host. *P. salamandra,* like all the digenetic trematodes, has a complex life history, but it deviates from the generalized life cycle primarily by the manner in which the miracidia are liberated.

The eggs of *P. salamandra* containing mature miracidia are ingested by a specific snail host in the digestive tract of which the eggs hatch. The liberated miracidia move through the intestinal wall and the developing larvae make their way to the hepatic gland (Owen, 1946) The life cycle then follows the generalized one. Though this deviation from the generalized cycle (i.e., in which the eggs hatch in the water and the miracidia penetrate the body of the snail) is not peculiar to *Plagitura salamandra,* studies on trematodes have not contributed materially to an understanding of the factor or factors influencing the liberation of miracidia in the intestinal tract.

A series of experiments was undertaken to attempt to hatch eggs of *P. salamandra in vitro,* and to ascertain, if possible, the factors necessary for hatching. These experiments were designed to determine the following:

1. The effect of extracts prepared from the digestive tracts of host specific snails and from non-specific aquatic snails on the hatching of eggs.
2. The effect of NaCl and KCl solutions of varying concentrations ranging in pH from 4.0–8.6 on the hatching of eggs.

MATERIALS AND METHODS

Preparation and examination of eggs.

Gravid trematodes obtained from the intestinal tract of newts were placed in 0.6% NaCl for 1 hour, then changed into tap water following which the eggs were rapidly discharged. Many of the eggs so obtained contained embryos and were kept in tap water for 4 to 5 days until actively motile miracidia were observed within the shell.

Forty to 50 eggs containing actively moving larvae were used in all experiments. The eggs (in 0.01 ml. of water) were placed in depression slides which contained 0.01 ml. of the various solutions to be tested. Solutions were replaced every 24 hours. All series were incubated in a moist chamber at room temperature (18°–21° C.) unless otherwise indicated. Eggs were examined daily for 5 days to determine hatching and motility of the liberated larvae. Hatching was recorded only when 20% or more of the eggs hatched.

[1] Contribution from the Miller School of Biology, University of Virginia. Part II of a dissertation submitted in candidacy for the Ph.D. degree.
Received for publication, January 6, 1954.

Preparation and testing of extracts of snail digestive tracts.

Aqueous extracts of digestive tracts were made from 25 of each of the following species of snails: *Pseudosuccinea columella* (Say), *Physella heterostropha* (Say), *Helisoma anceps* (Menke), *Oxytrema virginica* (Gmelin) (= *Goniobasis virginica*), *Nitocris carinata* (Brugiere). Digestive tracts were removed and ground with washed sterile sand in 0.5 ml. of triple distilled water. The homogenate, infused at 4° C. for 2–4 hours, was centrifuged and the supernatant fluid removed and used as the "extract." Immediately before using, each extract was divided into two equal portions and further diluted: one with an equal volume of distilled water and the second with an equal volume of 0.6% NaCl.

The eggs were placed in an aqueous extract and in one to which an equal volume of 0.6% NaCl (final concentration of NaCl = 0.3%) had been added. Controls of eggs in distilled water and 0.3% NaCl were maintained simultaneously.

Preparation and testing of the NaCl and KCl solutions.

Solutions of NaCl and KCl ranging in concentration from 0.1% to 1.0% were prepared in Clark and Lubs Buffers. The buffer solutions used for the NaCl dilutions had the following pH range: 4.0, 5.0, 7.0, and 7.6; while the KCl dilutions were made in buffers ranging from pH 4.0, 5.0, 7.0, 8.0, to 8.6. Similar concentrations were prepared with NaCl and KCl in monobasic and dibasic potassium phosphate buffers with a pH range of 5.0, 7.0, and 7.6. A Beckman model G meter was used for all pH determinations.

Eggs were placed in the buffered NaCl and KCl solutions and at the same time eggs were placed in the buffer solutions alone.

RESULTS OF EXPERIMENTS

Effect of extracts of snail digestive tracts.

The eggs maintained in the extract of *P. columella* hatched, as did also those eggs in the extract (0.3% NaCl) of three of the non-specific snails (Table 1). Al-

TABLE 1.—*Effect of digestive tract extracts of various snails, with and without the addition of NaCl on the hatching of eggs containing active miracidia at 18°–20° C.*

Snail Species	Aqueous Extract	NaCl & Extract
P. columella	2* M**	1M
P. heterostropha	–	1
H. anceps	–	1
O. virginica	–	3
N. carinata	–	–
Controls:	Distilled water	0.3% NaCl
	–	–

* Number refers to days of incubation before hatching.
** M indicates that some of the miracidia were motile after leaving egg shell.

though the extract of the non-specific snail plus the NaCl did result in hatching, none of these miracidia exhibited motility. The only motility observed was among some of those miracidia which were liberated in the extract of the host specific snail, *Pseudosuccinea columella.*

Effect of varying concentrations of NaCl in buffered solutions.

Some of the eggs hatched in all the NaCl solutions (Clark and Lubs Buffer) except in those with a pH of 4.0 and the one in which a 0.1% NaCl solution was buffered to a pH of 5.0 (Table 2). The eggs in concentrations of NaCl above 0.6%

TABLE 2.—*Effect of NaCl prepared in Clark and Lubs buffers and potassium phosphate buffers on the hatching of eggs containing active miracidia at 18°–21° C.*

NaCl Conc.	*Clark and Lubs Buffers*				*Potassium Phosphate Buffers*		
	pH 4.0	pH 5.0	pH 7.0	pH 7.6	pH 5.0	pH 7.0	pH 7.6
0.1%	–	–	3*M**	2 M	–	–	–
0.2%	–	3 M	3 M	2	–	–	–
0.3%	–	3 M	3 M	3	–	–	–
0.4%	–	3 M	3 M	3	–	–	–
0.5%	–	3	2 M	3	–	–	–
0.6%	–	3	2 M	3	–	–	–
0.7%	–	3	2	3	–	–	–
0.8%	–	3	2	3	–	–	–
0.9%	–	3	2	3	–	–	–
1.0%	–	3	2	3	–	–	–
Controls: Buffer only		–	2	–	–	2	–

* Number refers to days required for eggs to hatch.
** M indicates that some of the miracidia were motile after hatching.

hatched, but there was no apparent motility of the miracidia. Similarly, concentrations with a pH of 7.6, with the exception of the 0.1% NaCl dilution seemed to effect the release of non-motile miracidia. The control with the buffer solution having a pH of 7.0 resulted in the hatching of the eggs. Some of the eggs which failed to hatch in the high concentrations of NaCl appeared to be plasmolyzed.

At the end of 5 days the potassium phosphate buffer dilutions failed to effect hatching, though the buffer control having a pH of 7.0 did effect hatching in 2 days.

Effect of varying concentrations of KCl in buffered solutions.

The effective pH range, in the presence of KCl, for the liberation of miracidia shifted toward the less acid concentration (Table 3). The data show that 0.7% KCl at a pH of 7 using potassium phosphate buffer effected the escape of the miracidia most quickly. The miracidia liberated in this solution were more actively motile than were those in any of the other solutions used. Some of the eggs hatched in the potassium phosphate buffer control having a pH of 7.0. Here, as in the controls in the previous experiments, the liberated miracidia appeared to be non-motile.

CONCLUSION

It has been possible to effect the hatching of the eggs of *Plagitura salamandra* by means of NaCl and KCl solutions having certain pH ranges at room temperature.

TABLE 3.—*Effect of KCl prepared in Clark and Lubs buffers and in potassium phosphate buffers on the hatching of eggs containing active miracidia at 18°–21° C.*

KCl Conc.	*Clark and Lubs Buffers*					*Potassium Phosphate Buffers*		
	pH 4.0	pH 5.0	pH 7.0	pH 8.0	pH 8.6	pH 5.0	pH 7.0	pH 7.6
0.1%	–	–	–	–	4*	–	–	–
0.2%	–	–	4	–	4	–	–	2 M
0.3%	–	–	3 M**	3	3	–	–	2 M
0.4%	–	–	3 M	3	4	–	–	4
0.5%	–	–	5	3	3 M	–	–	2 M
0.6%	–	–	5	3	4 M	–	–	2 M
0.7%	–	–	4 M	4 M	4 M	–	1 M	4 M
0.8%	–	–	4 M	4 M	4 M	–	3 M	4 M
0.9%	–	–	5	4	4	–	3	4
1.0%	–	–	5	4	4	–	3	4
Controls: Buffer only	–	–	2	–	–	–	2	–

* Number refers to days required for eggs to hatch.
** M indicates that some of the miracidia were motile after hatching.

The mechanism utilized in the liberation of the miracidia from the trematode eggs has not yet been determined.

SUMMARY

1. Mature miracidia of *Plagitura salamandra* were hatched within 48 hours in the presence of intestinal tract extract of the host specific snail, *Pseudosuccinea columella;* the addition of NaCl to the extract effected hatching of motile miracidia in 24 hours.

2. Aqueous extracts of 3 species of aquatic snails resulted in the liberation of the mature miracidia only after the addition of NaCl. These miracidia, however, did not exhibit motility.

3. A study of the results obtained from exposing the eggs containing miracidia to buffered concentrations of NaCl and KCl from 0.1%–1.0%, having pH range of 4.0–8.6, permit the following statements:

a. Eggs of *Plagitura salamandra* containing mature miracidia hatched in a range of concentrations of NaCl with pH from 5.0–7.6 and KCl having pH from 7.0–8.6.

b. The released miracidia did not appear motile in all the solutions.

c. Hatching was observed in buffer solutions having a pH of 7.0

ACKNOWLEDGMENT

I wish to express my sincere appreciation to Professor B. D. Reynolds for his many helpful suggestions and the interest he has shown in this study.

Bibliography

Holl, F. J. 1928 A new trematode from the newt, *Triturus viridescens.* J. Elisha Mitchell Scient. Soc. **43:** 181–183.

Owen, H. M. 1946 The life history of *Plagitura salamandra* Holl, 1928 (Trematoda: Plagiorchiidae). J. Parasit., **32:** 553–562.

THE OCCURRENCE OF THE COCCIDIAN EIMERIA BUKIDNONENSIS TUBANGUI 1931, IN NIGERIAN CATTLE

Robert Pearson Lee

Veterinary Research Laboratory, Vom, Northern Nigeria

During the course of routine examinations a number of unusual coccidia oöcysts were found in the feces of a Fulani calf from Kagoro, Zaria Province. The parasites were scarce but 60 were recovered for detailed morphological examination and a few were available for sporulation tests. They were subsquently identified as *Eimeria bukidnonensis.* There are relatively few references to this parasite in the literature and as it appears to be the first time the species has been described from Nigeria it is considered desirable to record the findings in detail.

Eimeria bukidnonensis was originally described by Tubangui (1931) from the feces of a bull brought to Manila, from Bukidnon, Mindanao. Twenty-five oöcysts were examined. It was subsequently observed by Yakimov, Gusev, and Rastegaeva (1932) in Usbekistan and by Marčenko (1937) in North Caucasia.

It seems likely that the parasite occurs in Brazil since Yakimov (1936) found it

Received for publication, January 6, 1954.

TABLE 1.—*Dimensions of* Eimeria bukidnonensis *recorded by various workers*

Measurements in microns	Kagoro oöcysts (present author)	Mindanao oöcysts (Tubangui, 1931)	Alabama oöcysts (Christensen, 1941)	New York oöcysts (Christensen, 1938)
Mean length	44.0	48.6	36.6	..
Range in lengths	41.2–47.9	46.8–50.4	33–41	32–41
Mean width	32.1	35.4	26.7	..
Range in width	29.9–34.0	33.3–37.8	24–28	24–30
Mean shape index	1.37	1.37	1.37	..
Range in shape indices	1.27–1.45	..	1.25–1.56	..
No. of oöcysts measured	60	25	80	10

in 1 of 49 bovines imported from Sao Paulo to Leningrad. The first records of its recognition in North America appear to have been made by Christensen (1938) and Baker (1938), 10 oöcysts being examined from the same occurrence of the species. The next report of its occurrence known to the present author is by Christensen (1941) in which he described 80 oöcysts found in the feces of 3 bovines in Alabama.

MATERIALS AND METHODS

In the present examinations the öocysts were removed from the rectum manually and were placed with ice in a thermos flask for transportation to the laboratory. They remained on ice until required for examination. Oöcysts were recovered by passing a watery suspension of feces through a fine-mesh sieve and the filtrate was concentrated and washed by centrifuging. Concentrated sugar solution was added to the final deposit and the mixture was again centrifuged to bring the oöcysts to the surface. A drop was removed by means of a platinum loop and transferred to a cover slip which was lowered onto a hollow ground slide. In this way oöcysts floated free under the surface of the cover slip and were therefore not subjected to any pressure liable to cause distortion. Examinations and measurements were made under oil immersion by means of an ocular micrometer.

Sporulation was attempted in 2% potassium dichromate in a shallow watch glass at room temperature and sporulating oöcysts were examined daily.

FINDINGS

The oöcysts were readily distinguished from other coccidia in the preparations by their striking yellowish-brown color. They were pyriform and had a thick wall of approximately 2 microns in transverse section. The wall was of a darker color than the remainder of the oöcysts and invariably showed marked radial striations.

The micropyle was distinct and varied from 3.5 to 4.0 microns in diameter. The

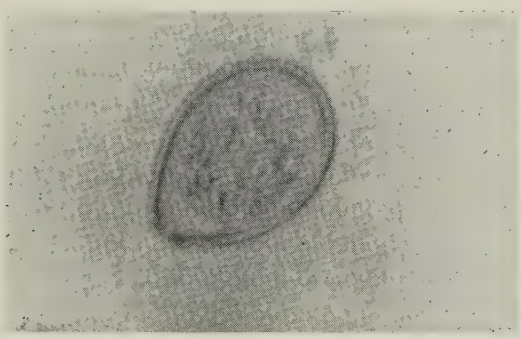


FIGURE 1. Unsporulated oöcyst of *Eimeria bukidnonensis,* approximately 32 microns wide. (Under oil immersion.)

other dimensions are shown in Table 1 together with the measurements recorded by the other workers referred to in this paper for comparison.

Only a few oöcysts were available for sporulation. Sporozoites in an advanced state of development were observed on the 17th day but, unfortunately, they were lost and no further material was obtainable. It is interesting to note that Baker (1938) observed the sporulation period to be 24–27 days while Christensen obtained complete sporulation in 4 to 7 days.

Since these examinations were made oöcysts morphologically similar to the above have been seen in cattle feces submitted to this laboratory from widely scattered areas of Northern Nigeria. Unfortunately, all these samples were stored during transit in formalin and it was therefore impossible to carry out sporulation tests.

ACKNOWLEDGEMENTS

I am indebted to the Inspector-General of Animal Health Services, Nigeria, and to the Assistant Director of Laboratory Services for permission to publish this note and to Mr. R. A. O. Shonekan, Laboratory Technician, for technical assistance.

REFERENCES

Baker, D. W. 1938 Observations on *Eimeria bukidnonensis* in New York State Cattle. J. Parasit. **24** (6, Suppl.) : 15–16.

Christensen, J. F. 1938 Occurrence of the coccidian *Eimeria bukidnonensis* in American cattle. Proc. Helminth. Soc. Washington **5**: 24.

———1941 The oöcysts of coccidia from domestic cattle in Alabama (U.S.A.), with descriptions of two new species. J. Parasit. **27**: 203–220.

Marčenko, C. F. 1937 Kokcidiozy domašnih zivotnyh na severnom Kavkaze. Sborn Rab. Leningrad. vet Inst. 1937, pp. 155–159. Abstract in Vet. Bull. **9**: 80.

Tubangui, M. A. 1931 *Eimeria bukidnonensis,* a new coccidium from cattle, and other coccidial parasites of domesticated animals. Philippine J. Sci. **44**: 253–271.

Yakimov, V. L., Gusev, V. F., and Rastegaeva, E. F. 1932 Zur Frage der Kokzidien der Rinder in Usbekistan (Mittelasien). Zentralbl. Bakteriol. Abt. Orig. **126**: 111–118.

———1936 Coccidios dos animaes domesticos do Brasil. Arch. Inst. biol. Def. Agric. anim. S. Paulo **7**: 167–187. Abstract in Vet. Bull. **3**: 281.

CAMBARINCOLA GRACILIS SP. NOV., A BRANCHIOBDELLID OLIGOCHAETE COMMENSAL ON WESTERN CRAYFISHES

Dorothy A. Robinson

Department of Biology, Lewis and Clark College, Portland, Oregon

While studying the branchiobdellids from western crayfishes, an undescribed species of *Cambarincola* was found on crayfishes collected at a number of localities. This new species, for which the name *Cambarincola gracilis* is proposed, is similar to both *C. macrodonta* Ellis, 1912 and *C. inversa* Ellis, 1919, but may be differentiated from these on the basis of the structure of the head, jaws, and accessory sperm tube.

I wish to express my appreciation to Dr. Eugene N. Kozloff of Lewis and Clark College for his guidance and encouragement throughout the progress of this study. Gratitude is also extended to Dr. Fenner A. Chace, Jr. for the loan of paratypes of *Cambarincola inversa* in the United States National Museum.

METHODS

The worms were taken from crayfish which had been preserved in higher grades of ethyl and isopropyl alcohol. The most satisfactory whole mounts were prepared

Received for publication, December 18, 1953.

by progressive staining with Delafield's alum hematoxylin. Unstained specimens and specimens stained with borax carmine were also used, but these were in general less suitable for morphological study. Sagittal and transverse serial sections of worms embedded in paraffin were stained with Delafield's hematoxylin.

Cambarincola gracilis sp. nov.

The general outline and external appearance of this species (Fig. 1) is quite similar to that of *C. inversa,* described by Ellis (1919). The body is elongate and terete, becoming gradually wider from segment I to segment VI, the widest segment. The posterior segments taper rapidly to the terminal acetabulum. The length of sexually mature worms ranges from about 2 mm. to about 4 mm. The width, at segment VI, of a specimen about 2 mm. long is approximately 0.4 mm.

The head is approximately as long as the first 3 body segments combined. Its width is slightly greater than that of segment I. The lips are separated from the head by a constriction, and the jaws are located in this narrowed region. There are 2 lips, both entire, the upper being slightly longer than the lower. The pharynx has 2 diverticula, one dorsal, the other ventral. The dorsal diverticulum is slightly anterior to the ventral one.

The jaws are yellow brown. The dorsal jaw (Figs. 2a, 2b) bears 1 large median tooth and 2 weakly developed tubercles on the dental ridge. The ventral jaw (Figs. 2c, 2d) has 2 teeth which are smaller than the single tooth of the dorsal jaw, and 2 small, sharp tubercles on the dental ridge. The jaws of *C. gracilis* do not have the excavated and somewhat triangular base described for the jaws of *C. macrodonta* and *C. inversa.* The base, as seen in dorsal or ventral view, is rounded.

The reproductive organs are characteristic of the genus. There are 2 pairs of testes, a pair in segment V, the other pair in segment VI. The spermatheca is not bifid, and opens by a prominent pore on the ventral side of segment V. The bursa in segment VI (Figs. 3, 4) is conspicuous and eversible. The accessory sperm tube enters the genital atrium near the point where the latter joins the duct of the bursa. The vasa deferentia leading from the testes in segments V and VI enter the anterior portion of the genital atrium. There is a pair of ovaries in segment VII.

A list of the host species of crayfish together with the different locations from which *Cambarincola gracilis* was taken follows. *Pacifastacus klamathensis* (Stimpson) : **Washington**—Walla Walla Co. (campus of Whitman College, Walla Walla), Clallam Co. (tributary of Elwha River) ; **Oregon**—Multnomah Co. (Crystal Springs Creek, Portland), Clackamas Co. (Tryon Creek, near Oswego), Lincoln Co. (Yachats River 1 mile east of Yachats) ; **California**—Siskiyou Co. (Klamath River 1 mile west of mouth of Ash Creek ; same river 2 miles east of Hornbrook) ; *P. trowbridgii* (Stimpson) : **British Columbia**—Burdette Creek, (near Burnaby), Colquitz River (near Victoria) ; *P. gambelii* (Girard) : **Oregon**—Harney Co. (near OO Ranch Corral, Malheur National Wildlife Refuge).

The holotype, from *Pacifastacus klamathensis* collected by Dr. A. G. Rempel on the campus of Whitman College, Walla Walla County, Washington, has been deposited in the United States National Museum (U.S.N.M. No. 26110). The two paratypes (U.S.N.M. Nos. 26111, 26112) have been selected from the specimens taken from *P. klamathensis* collected by Mr. Harold Wolf in the Klamath River 1 mile west of the mouth of Ash Creek, Siskiyou County, California and from *P. trow-*

bridgii collected by G. Clifford Carl in Burdette Creek, near Burnaby, British Columbia.

DISCUSSION

Because *Cambarincola gracilis* is similar to *C. inversa* in general form, it is possible that some specimens of *C. gracilis* may have been referred to *C. inversa* by earlier authors. Goodnight (1940) recorded *C. inversa* from a number of stations in Oregon, Washington, and Idaho. Although I have examined crayfishes from widely scattered localities in northern California, Oregon, Washington, and British Columbia, I did not find branchiobdellids conspecific with the paratypes of *C. inversa.*

Cambarincola gracilis may be differentiated from *C. inversa* by the structure of its jaws and the organization of the accessory sperm tube. In *C. gracilis* the base of the jaws is rounded, whereas in *C. inversa* the base is excavated. In *C. inversa* there is a smaller tooth on either side of the large median tooth of the dorsal jaw, but in *C. gracilis* these lateral teeth are lacking. The ventral jaw of *C. gracilis* also lacks the lateral teeth present on the ventral jaw of *C. inversa.* In dorsal or ventral view, the jaws of *C. inversa* are somewhat triangular in outline, and are widest anteriorly.

The accessory sperm tube of *C. gracilis* is very unlike that of *C. inversa,* and more closely resembles the accessory sperm tube of *C. macrondonta,* described by Ellis (1912). It appears to be a relatively undeveloped structure, closely applied to the genital atrium and opening into the atrium at the point where the latter joins the duct of the bursa. In *C. inversa* the accessory sperm tube is large, and is apparently entirely free of the atrium except where it opens into the latter.

SUMMARY

Cambarincola gracilis sp. nov. is described from crayfishes referable to *Pacifastacus klamathensis, P. trowbridgii,* and *P. gambelii* collected at a number of localities in northern California, Oregon, Washington, and British Columbia. *C. gracilis* appears to be closely related to *C. inversa* Ellis and *C. macrodonta* Ellis.

Literature Cited

Ellis, M. M. 1912 A new discodrilid worm from Colorado. Proc. U. S. Nat. Mus. **42**: 481–486.

——— 1919 The branchiobdellid worms in the collections of the United States National Museum, with descriptions of new genera and new species. Proc. U. S. Nat. Mus. **55**: 241–265.

Goodnight, C. J. 1940 The Branchiobdellidae (Oligochaeta) of North American crayfishes. Ill. Biol. Monogr. **17** (3) : 75 pp.

Explanation of Plate

Cambarincola gracilis sp. nov.

The figures have been prepared with the aid of a camera lucida, and with the exception of Figs. 2b and 2d are oriented in such a way that the dorsal aspect is nearest the top of the page.

Abbreviations used: ast, accessory sperm tube; b, bursa; d, duct of bursa; ga, genital atrium; vd, vas deferens.

Fig. 1. Entire worm; lateral view from left.

Fig. 2a. Dorsal jaw; lateral view from left.

Fig. 2b. Dorsal jaw; dorsal view.

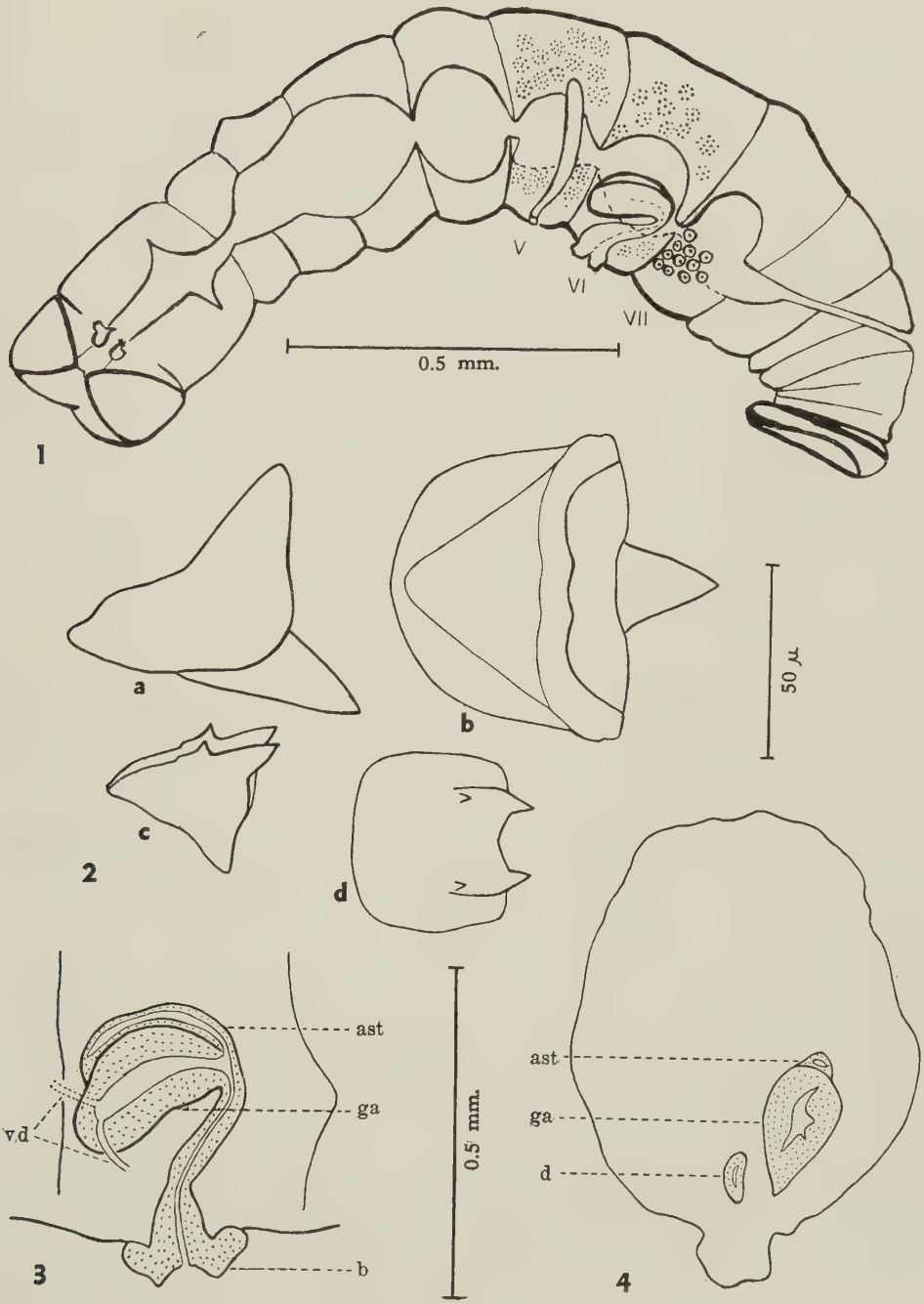
Fig. 2c. Ventral jaw; lateral view from left.

Fig. 3. Male reproductive organs in segment VI (testes omitted); semidiagrammatic, reconstructed from sagittal serial sections.

Fig. 4. Male reproductive organs in transverse section through posterior half of segment VI; semidiagrammatic.

Fig. 2d. Ventral jaw; dorsal view.

PLATE I



VENTRIDENS LIGERA, A NEW FIRST INTERMEDIATE HOST OF *PANOPISTUS PRICEI* SINITSIN, 1931 (TREMATODA: BRACHYLAIMATIDAE)[1]

JOHN B. VILLELLA

Biological Effects of Irradiation Laboratory, AEC, University of Michigan, Ann Arbor

In at least 9 of the experimentally determined life cycles of the BRACHYLAIMATINAE, from 3 to 9 species of mollusks have been recorded as second intermediate hosts of single brachylaimatid species. In the literature of the BRACHYLAIMATINAE reviewed a single brachylaimatid, viz. *Panopistus pricei,* has been reported to utilize more than 1 molluscan species as the first intermediate host. In this case 2 land snails, *Zonitoides arboreus* and *Agriolimax agrestis,* were reported as primary hosts.

Sinitsin (1931) briefly described a metacercaria found in only 1 of 138 specimens of *Gastrodonta suppressa* (now known as *Ventridens suppressus*). This metacercaria he considered to be the larval form of an adult brachylaimatid parasitic in the small intestine of the short-tailed shrew, *Blarina brevicauda* (Say). Krull (1935), believing that Sinitsin's evidence was inconclusive, determined experimentally that *Zonitoides arboreus* served as a first as well as a second intermediate host of *P. pricei.* Reynolds (1938), without controlled feeding experiments, described cercarial and metacercarial stages of this brachylaimatid from the slug, *Agriolimax agrestis,* in Virginia.

OBSERVATIONS

In a survey of the land snails of the Ann Arbor area during 1950–1952, metacercariae believed to be *P. Pricei,* were recovered from the heart and pericardial cavity of *Ventridens ligera, Stenotrema monodon, Zonitoides nitidus,* and a slug, *Deroceras laeve.* Of 3844 *Ventridens ligera* collected, 3 were shedding blunt-tailed brachylaimatid cercariae and about 50 percent of the snails were parasitized with brachylaimatid metacercariae. The location of the genital pore (behind the posterior testis) suggested that the metacercariae removed from the heart and pericardial cavity belonged to the genus *Panopistus* Sinitsin, 1931.

Sinitsin's original metacercaria of *P. pricei* from *Ventridens suppressus,* slide #28360, U. S. Nat'l. Mus. Helm. Coll., was loaned to the writer by Dr. E. W. Price for comparison with the metacercariae obtained from *Ventridens ligera* and other land snails, collected for the present study. An examination of the slide showed that 1 of 2 metacercariae was identical with the metacercariae from *Ventridens ligera, Stenotrema monodon, Zonitoides nitidus,* and *Deroceras laeve.* This suggested that Sinitsin correctly identified the metacercaria from *Ventridens suppressus* as a larval form of *P. pricei.* A second metacercaria on this slide, unidentified on the label, has been identified by the writer as *Entosiphonus thompsoni,* also described by Sinitsin (1931). Since multiple infections of brachylaimatids are quite common in land snails. *P. pricei* and *Entosiphonus thompsoni* probably were

Received for publication, June 11, 1953.

[1] Appreciation is expressed to Dr. A. E. Woodhead, Department of Zoology, under whom this work was done. Contribution from the Department of Zoology, University of Michigan.

recovered from the same snail. I have frequently observed *P. pricei* associated with other species of brachylaimatids within the same snail host.

EXPERIMENTS AND RESULTS

To verify the identity of this brachylaimatid, the life cycle was carried out under laboratory conditions. Of 86 short-tailed shrews collected from areas known to have snails infected with brachylaimatid metacercariae, 42 were infected with mature and immature adults of *P. pricei.* Eggs from mature worms were then fed to laboratory-raised *Ventridens ligera, Stenotrema monodon, Zonitoides nitidus,* and *Deroceras laeve.* Branching sporocysts developed only in *Ventridens ligera,* producing cercariae identical with the cercariae observed in naturally-infected *Ventridens ligera.* Cercariae were produced in 44–50 days following exposure of the snails to the eggs of *P. pricei.* Measurements in millimeters of various structures of 25 cercariae from *Ventridens ligera* (fixed in hot 10 percent formalin) were as follows: body length, including tail 0.184 to 0.272 (av. 0.225); width 0.067 to 0.102 (av. 0.082); oral sucker 0.028 to 0.058 (av. 0.049) by 0.046 to 0.058 (av. 0.052); pharynx 0.018 to 0.025 (av. 0.021) by 0.018 to 0.023 (av. 0.022); acetabulum 0.037 to 0.051 (av. 0.045) by 0.041 to 0.058 (av. 0.050). These values are essentially within the range of dimensions listed by Krull for the cercariae from *Zonitoides arboreus.* The number (7 pairs) and arrangement of the flame cells and the general morphology of the cercaria from *Ventridens ligera* agree with similar data for the cercaria from *Zonitoides arboreus,* as reported by Krull.

Cercariae from laboratory-raised *Ventridens ligera* were transferred to laboratory-raised second intermediate hosts. Metacercariae, identical with those in natural infections, were recovered from the laboratory-raised snails 75–90 days following exposure of the snails to cercariae. Measurements in millimeters of various structures of 25 metacercariae from *Ventridens ligera* (fixed in hot 10 percent formalin, stained and mounted) were as follows: body length 0.775 to 0.857 (av. 0.825); width 0.272 to 0.340 (av. 0.311); oral sucker 0.136 to 0.197 (av. 0.185) by 0.156 to 0.197 (av. 0.166); pharynx 0.068 to 0.095 (av. 0.083) by 0.082 to 0.102 (av. 0.092); acetabulum 0.136 to 0.190 (av. 0.171) by 0.143 to 0.190 (av. 0.168); anterior testis 0.054 to 0.075 (av. 0.063) by 0.041 to 0.061 (av. 0.052); posterior testis 0.048 to 0.068 (av. 0.056) by 0.034 to 0.045 (av. 0.042); ovary 0.034 to 0.045 (av. 0.040) by 0.034 to 0.048 (av. 0.044). Several of the foregoing dimensions do not agree with those listed by Krull for the metacercariae of *P. pricei* from *Zonitoides arboreus.* However, differences may be due to the method of fixation, state of contraction, or possibly to the age of the metacercariae.

The time required for the development of the intramolluscan stages in *Ventridens ligera* agrees essentially with the time reported by Krull for similar stages of *P. pricei* from *Zonitoides arboreus.*

Because of the difficulty of raising the short-tailed shrew in the laboratory, 75–90 day old metacercariae of *P. pricei* were fed to 3 albino rats, 3 albino mice, 2 domestic rabbits, and 1 young cottontail rabbit. Adults of *Panopistus* were never recovered from these hosts.

SUMMARY

This study indicates that *Ventridens ligera* is a new, and the third recorded, first intermediate host of *P. pricei,* a brachylaimatid trematode parasitic in the small

intestine of the short-tailed shrew. *Zonitoides arboreus* and the land slug, *Agriolimax agrestis,* had been reported previously as first intermediate hosts of this brachylaimatid. Further, Sinitsin apparently recognized and correctly identified the metacercaria from the pericardial chamber of *Ventridens suppressus* as an intramolluscan stage of this parasite, and Krull demonstrated that *Zonitoides arboreus* may also serve as an experimental second intermediate host. To these the present study adds 4 previously unreported snail species, *Zonitoides nitidus, Stenotrema monodon, Ventridens ligera,* and *Deroceras laeve,* making a total of 6 snail species that serve as second intermediate hosts.

The utilization of 3 species of land snails as primary hosts by *Panopistus pricei* indicates a lack of specificity for the first intermedate host, as well as for the second intermediate host.

Literature Cited

Krull, W. H. 1935 Studies on the life history of *Panopistus pricei* Sinitsin, 1931 (Trematoda). Parasitology **27**: 93–100.

Reynolds, B. D. 1938 Developmental stages of *Panopistus pricei* Sinitsin in *Agriolimax agrestis.* Parasitology **30**: 320–323.

Sinitsin, D. 1931 Studien über die Phylogenie der Trematoden. V. Revision of *Harmostominae* in the light of new facts from their morphology and life history. Zeitschr. Parasitenk. **3**: 786–835.

RESISTANCE OF A PYRIMETHAMINE–RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM* TO CERTAIN 2,4–DIAMINOPYRIMIDINES AND RELATED COMPOUNDS

Joseph Greenberg and Howard W. Bond

U. S. Department of Health, Education, and Welfare
Public Health Service
National Institutes of Health, National Microbiological Institute,
Laboratory of Tropical Diseases, Bethesda 14, Maryland

Strains of several species of malaria made resistant to pyrimethamine, 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine, have been found to be resistant to chlorguanide (*Plasmodium cynomolgi,* Schmidt and Genther, 1952; *P. vivax,* Hernandez, Myatt, Coatney and Jeffery, 1953; *P. berghei,* Thurston, 1953). Strains of parasites resistant to chlorguanide sometimes are (Robertson, Davey and Fairley, 1952; Jaswant Singh, Ray, Basu and Nair, 1952; Jones, 1953) and sometimes are not resistant to pyrimethamine (Rollo, 1951; Wilson and Edeson, 1953). We found that a strain of *P. gallinaceum* resistant to chlorguanide was not resistant to 2,4-diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine (Greenberg and Richeson, 1951). Schmidt (personal communication) observed a strain of *P. cynomolgi* resistant to pyrimethamine that was not resistant to the 5-*p*-chlorophenoxy analogue.

There are many reasons for believing that the modes of action of 2,4-diaminopyrimidines and chlorguanide are similar. The failure to obtain cross-resistance would imply that resistance is associated with changes in the parasite involving particular portions of the molecule. To locate the portion of the molecule involved, a limited series of diaminopyrimidines, a diaminotriazine, and chlorguanide were tested against a strain of *P. gallinaceum* made resistant to pyrimethamine.

Received for publication, December 7, 1953.

MATERIALS AND METHODS

The pyrimethamine-resistant strain of *P. gallinaceum* used in these experiments was obtained by the usual procedure of exposing the parasite to increasing amounts of the drug over a period of time. The drug was administered at minimal effective doses, twice daily for 4 days beginning 4 to 5 hours before inoculation of parasites. When the mean parasitemia of the treated chicks, examined on day four, significantly exceeded 25 per cent of the controls, the dosage of drug administered in the next passage was doubled. A three-day drug-free period usually intervened between passages. Treatment was discontinued when it was found that 64 times as much pyrimethamine was required to suppress the resistant strain as was required for the parent strain. At the time the present experiments were carried out the resistant strain had not been exposed to drug for about 6 months.

Week-old New Hampshire Red chicks, weighing 45 to 60 grams, served as hosts. The methods of inoculating parasites, determination of parasitemia, and criteria for minimal effective dose have been described elsewhere (Coatney, Cooper, Eddy and Greenberg, 1953). Briefly, the chicks were inoculated intravenously with 16 million parasitized erythrocytes. They were treated twice daily with the test compounds for 4 days beginning 4 to 5 hours before inoculation. Parasitemia was determined on the morning of the fifth day. The minimum effective dose of a compound is that amount which reduced the parasitemia of the treated chicks to at least one-fourth of that of the untreated controls. Five chicks were used for each dosage regimen, and for each series 10 chicks served as untreated controls.

All of the pyrimidines except the diacetyl derivative of pyrimethamine were kindly furnished by Burroughs Wellcome and Company (U.S.A.), Inc., chlorguanide and the triazine by Imperial Chemical Industries. The diacetyl derivative of pyrimethamine was synthesized by one of us (H.W.B.).

RESULTS

The results are summarized in the table. The treated strain was 64-fold resistant to pyrimethamine and to the diacetyl derivative of pyrimethamine. It was 512-fold resistant to the 6-amyl analogue (DR 16,093) of pyrimethamine; it was

TABLE 1.—*The effect of diaminopyrimidines, chlorguanide, and a diaminotriazine on a pyrimethamine-resistant strain of* Plasmodium gallinaceum

Compound	Minimum effective dose (mg./gm.)		
	Parent strain (P)	Resistant strain (R)	R/P
DR 16,056	0.00004	0.00256	64
DR 16,311	0.00008	0.00128	16
DR 16,093	0.0000625	0.032	512
DR 16,279	0.00004	0.0025	64
DR 16,071	0.001	0.008	8
DR 16,092	0.001	0.002	2
DR 15,787	0.0078	0.0156	2
SN 12,837	0.002	0.032	16
DR 16,182	0.00002	0.00016	8

DR 16,056—Pyrimethamine : 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine
DR 16,311—2,4-Diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine
DR 16,093—2,4-Diamino-5-(*p*-chlorophenyl)-6-amylpyrimidine
DR 16,279—2,4-Diacetylamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine
DR 16,071—2,4-Diamino-5-(*p*-chlorobenzyl)-6-methylpyrimidine
DR 16,092—2,4-Diamino-5-(*p*-nitrobenzyl)-6-methylpyrimidine
DR 15,787—2,4-Diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine
SN 12,837—Chlorguanide : N_1-(*p*-chlorophenyl)-N_5-isopropylbiguanide
DR 16,182—4,6-Diamino-1-(3′,4′-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine

relatively less resistant to the 6-methyl analogue (DR 16,311) though the reduction in activity might be attributable to the second chlorine on the 5-phenyl ring.

The strain was relatively less resistant to pyrimidines in which the linkage between the 5-phenyl ring and the pyrimidine nucleus was altered. The strain was only 8-fold resistant to the 5-benzyl-6-methyl analogue (DR 16,071) of pyrimethamine; and when the chlorine of this compound was replaced with a nitro group (DR 16,092) resistance almost disappeared. The pyrimethamine-resistant strain was almost as sensitive as the parent strain to the 5-(*p*-chlorophenoxy)-6-methyl analogue (DR 15,787) of pyrimethamine.

The pyrimethamine-resistant strain was about 16-fold resistant to chlorguanide (SN 12,837) and about 8-fold resistant to the triazine (DR 16,182) which is reported to be the active metabolic product of the dichlorophenyl analogue of chlorguanide (Crowther and Levi, 1953).

DISCUSSION

It is apparent that resistance to pyrimethamine is not always associated with equal resistance to related compounds. There appears to be a rough correlation between the degree of resistance to a compound and the activity of the compound against the parent strain. Looked at another way, certain changes in the substituents of the pyrimidine molecule produce relatively larger changes in the degree of activity against the parent strain of the parasite than do others. For example, the manner in which the phenyl group is attached to the pyrimidine ring influences antimalarial activity more than changes in the 6-position substituent. The changes in the molecule which appear to make the largest changes in the degree of activity of the compound appear to make the largest differences in the degree of resistance of the pyrimethamine-resistant strain. (The 6-amyl compound, DR 16,093, is an obvious exception.) This suggests that resistance is related to focal points in the molecule with, perhaps, the manner of attachment of the phenyl group to the pyrimidine ring being the most important. The degree of resistance becomes increasingly smaller as one proceeds from the 5-phenyl compounds, through the 5-benzyl compounds, to the 5-phenoxy compound. The lower degree of resistance of the triazine may be considered to be related to the fact that the phenyl group is attached through a ring nitrogen. The tentative relationships suggest that development of resistance in the organism to these pyrimidines may be accomplished by changes in an "acceptor" molecule in the parasite, rather than by the development of a metabolic shunt mechanism. It is also likely that, if such changes occur, they do so in small steps, since the degree of resistance to pyrimethamine presumably can be maintained at any point by withdrawing the parasite from exposure to the drug.

These tentative conclusions point to several questions, among them being: (1) do the changes which produce resistance always occur in the same direction or in the same order? (2) do all strains of a parasite which have acquired the same degree of resistance to one diaminopyrimidine respond the same to related compounds?

There are many unexplained facts about resistance and cross-resistance. Parasites which are resistant to pyrimethamine are usually somewhat resistant to chlorguanide (see introduction); but the converse is not always true. (The chlor-

guanide-resistant strain maintained in this Laboratory is not significantly resistant to pyrimethamine.) Our pyrimethamine-resistant strain, as reported in this paper, is 16-fold resistant to chlorguanide. Neither of these strains is significantly resistant to the 5-(*p*-chlorophenoxy) pyrimidine (DR 15,787). And, finally, we have not been able to develop a strain resistant to the last compound.

SUMMARY

Seven diaminopyrimidines (including pyrimethamine), chlorguanide, and a diaminotriazine were tested against a pyrimethamine-resistant strain of *Plasmodium gallinaceum*. The strain was 64-fold resistant to pyrimethamine. It showed relatively little resistance (2-fold) to some chemically related compounds and marked cross-resistance (64- to 512-fold) to others. It was about 16-fold resistant to chlorguanide and 8-fold resistant to a diaminotriazine related to chlorguanide.

REFERENCES

COATNEY, G. R., COOPER, W. C., EDDY, N. B., AND GREENBERG, J. 1953 Survey of Antimalarial Agents, Public Health Monograph No. 9, U. S. Government Printing Office, Washington, D C.

CROWTHER, A. F. AND LEVI, A. A. 1953 Proguanil—The isolation of a metabolite with high antimalaria activity. Br. J. Pharmacol. & Chemotherapy **8**: 93–97.

GREENBERG, J. AND RICHESON, E. M. 1951 Effect of 2,4-diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine and 2,4-diamino-6,7-diphenylpteridine on a chlorguanide-resistant strain of *Plasmodium gallinaceum*. Proc. Soc. Exper. Biol. & Med. **77**: 174–176.

HERNANDEZ, T., MYATT, A. V., COATNEY, G. R., AND JEFFERY, G. M. 1953 Studies in human malaria. XXXIV. Acquired resistance to pyrimethamine (Daraprim) by the Chesson strain of *Plasmodium vivax*. Am. J. Trop. Med. & Hyg. **2**: 797–804.

JASWANT SINGH, RAY, A. P., BASU, P. C., AND NAIR, C. P. 1952 Acquired resistance to proguanil in *Plasmodium knowlesi*. Trans. Roy. Soc. Trop. Med. & Hyg. **46**: 639–649.

JONES, S. A. 1953 Experiments to determine if a proguanil-resistant strain of *P. falciparum* would respond to large doses of pyrimethamine. Brit. M. J. (4817) : 977.

ROBERTSON, G. I., DAVEY, D. G., AND FAIRLEY, N. H. 1952 Cross-resistance between "Daraprim" and proguanil. Brit. M. J. (4796) : 1255–1256.

ROLLO, I. M. 1951 A 2,4-Diaminopyrimidine in the treatment of proguanil-resistant laboratory malarial strains. Nature **168**: 332.

SCHMIDT, L. H. AND GENTHER, C. S. 1952 The antimalarial properties of 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (Daraprim). J. Pharm. & Exper. Therap. **107**: 61–92.

THURSTON, J. P. 1953 *Plasmodium berghei*. Exper. Parasit. **2**: 311–332.

WILSON, T. AND EDESON, J. F. B. 1953 Treatment of acute malaria with pyrimethamine. Brit. M. J. (4804) : 253–255.

FURTHER STUDIES ON THE HYBRIDIZATION OF STRAINS OF *PLASMODIUM GALLINACEUM*

HELEN LOUISE TREMBLEY AND JOSEPH GREENBERG

U. S. Department of Health, Education, and Welfare, Public Health Service
National Institutes of Health
National Microbiological Institute[1]
Bethesda, Maryland

The BI strain of *Plasmodium gallinaceum* does not produce normal erythrocytic parasites when passaged through the mosquito (Haas, Wilcox, Ewing and Coleman, 1948; Trembley, Greenberg and Coatney, 1951; and Greenberg, Trembley and Coat-

Received for publication, January 26, 1954.

[1] Laboratory of Tropical Diseases.

ney, 1953). The chicks die of late exoerythrocytic parasites, with a few, non-pigmented parasites detectable in the erythrocytes just prior to death. The strain can normally undergo only one transfer through the mosquito without the intervention of the investigator. If the latter can keep the chicks alive by therapy (Greenberg *et al.,* 1953) or keep the parasite alive by the serial subinoculation of blood (Lewert, 1948, 1950; Haas *et al.,* 1948), the strain eventually regains its ability to produce normal erythrocytic parasites and is again infectious to the mosquito. The M strain, on the contrary, is benign and produces no detectable late exoerythrocytic forms when passaged through the mosquito (Hass *et al.,* 1948; Trembley, Greenberg and Coatney, 1951a). Dr. Clay G. Huff was kind enough to examine tissues of chicks infected with the M strain parasites and found late tissue forms in serial sections of brain and other tissues. We have not observed such forms in routine examinations of brain smears made at autopsy; and, since the mortality rate of this strain approaches zero percent, such forms obviously do not contribute to the death of the chicks. Conversely, the BI strain when mosquito-passaged produces abundant phanerozoites, readily detectable in brain smears and causing a mortality of 100 percent.

In an earlier experiment (Greenberg and Trembley, 1953) chicks were inoculated intravenously with equal numbers of erythrocytic forms of both strains of the parasite. Mosquitoes were fed on these chicks, and the sporozoites from these mosquitoes were used to infect other chicks. The resulting infections (F_1) in these chicks had both erythrocytic and exoerythrocytic parasites, indicating that the characteristics of both strains were present. Mosquitoes were fed on the chicks harboring the F_1 infections, and the sporozoites from these mosquitoes produced infections (F_2) in no way distinguishable from the parent M strain infections. The F_2 infections were benign and devoid of late tissue forms. This pattern of infection persisted through another mosquito passage (F_3).

The following possible explanations were offered to explain these results: (1) no hybridization occurred; (2) hybridization occurred but the genes producing the BI strain characteristics were dominant and the hybrids behaved as the parent BI strain, i.e., only one mosquito passage was possible without the intervention of the investigator; (3) the hybrids all behaved as the parent M strain because of a cytoplasmically inherited factor which, for example, limited reproduction in the fixed tissue.

It was felt that more information could be obtained if the strains were exposed to the possibility of two successive hybridizations. This could be done conveniently by treating the F_1 infections with pyrimethamine, which prevents death from tissue forms (Greenberg, Trembley and Coatney, 1953). When chicks infected with BI strain sporozoites only were treated at about day 7 after inoculation, normal erythrocytic parasites were detected after day 18, and the strain was infectious to mosquitoes after day 20. When sporozoite-induced M strain infections were treated similarly, the course of the infection after treatment was about the same as that of the BI strain, i.e., the strain was infectious after day 20 (unpublished data). In an attempt to bring about a second hybridization (?), chicks carrying the F_1 generation of hybrid (?), M × BI, infections were treated with pyrimethamine on or about the seventh day after inoculation, and mosquitoes were allowed to feed on or after day 20.

MATERIALS AND METHODS

Week-old New Hampshire Red chicks weighing 45 to 60 grams were used. The vector was laboratory-reared *Aedes aegypti.* Two strains of *P. gallinaceum,* M and

BI, were used; their principal characteristics are described in the first paragraph of this paper. One mosquito equivalent of sporozoites was inoculated according to the method recommended by Coatney, Cooper and Trembley (1945). The methods of inoculating blood and of making and reading blood smears for parasitemia have been described by Coatney, Cooper, Eddy and Greenberg (1953). Blood smears were read daily or every other day beginning on day 7 after the inoculation of sporozoites. Brain smears of all chicks were made at autopsy and examined for tissue forms of the parasite (Coatney *et al.*, 1945). Pyrimethamine was administered orally by catheter in dilute lactic acid.

RESULTS

Heparinized blood from a donor chick infected by sporozoites with the M strain and from a donor chick infected by blood passage with the BI strain of *P. gallinaceum* was diluted with physiological saline and pooled so that approximately equal numbers of parasites of each strain were present in the preparation (8×10^6 parasitized erythrocytes per 0.1 ml.). Each of 10 chicks was inoculated with 0.1 ml. of this mixture. Of these, 3 chicks were selected, 2 on day 7 and 1 on day 8, each 1 to act as host for feeding a single lot of 200 mosquitoes. Eleven and 12 days later sporozoites from each of the 3 lots of mosquitoes were used to infect 10 chicks for a total of 30. On day 9 of the infection, each chick of the latter group was treated with a single dose (0.016 mg./gm.) of pyrimethamine. Three chicks died of exoerythrocytic infections before treatment, and 4 more chicks died from the same cause one and two days after treatment. When the remaining chicks were killed 24 to 26 days after inoculation, 15 to 17 days after drug, 4 more chicks exhibited rare exoerythrocytic parasites in smears of the brain. Therefore, 11 of the 30 chicks showed evidence of late tissue forms, there being at least 2 from each of the 3 groups.

Mosquitoes were fed on 1 or 2 chicks from each group on the twentieth, twenty-first, and on the twenty-fourth day after inoculation. Ten to 14 days later sporozoites from mosquitoes of each lot were used to infect 10 chicks; a total of 30. Three of these chicks died on day 4 of causes unknown. One chick died on day 15, probably of parasitemia (35 percent on day 14); no tissue forms were detectable. The remaining chicks survived to day 21 when they were killed. All the chicks which survived to day 21 exhibited a normal parasitemia; none had tissue forms in smears of the brain made at autopsy.

DISCUSSION

Following the initial inoculation of infected blood, these experiments were done in triplicate in that 3 independent passage lines were maintained throughout. The results in each of the 3 lines were essentially the same. As in our earlier experiment (Greenberg and Trembley, 1953) the characteristics of the BI strain were present after the first potential hybridization. Seven chicks died of exoerythrocytic infections just before or just after treatment with pyrimethamine and 4 additional chicks exhibited rare tissue forms when autopsied during the fourth week of the infection. None of the chicks used as donors for the F_2 infection had brain forms at autopsy. The ability to produce late tissue forms disappeared after the parasites were allowed a second possible hybridization by passage through mosquitoes. It should be noted that when the BI strain alone was treated similarly the strain characteristics persisted without change through 4 consecutive mosquito transfers (Greenberg *et al.*, 1953).

If there were no hybridization, or if the BI strain characteristics were genetic, dominant or recessive, one would expect that the relative proportions of the 2 strains would remain constant for an indefinite series of passages. This would be true, even if there were a disproportion in the rate of gametocyte production between the 2 strains. The second serial mosquito passage of the mixed-strain infections would be essentially like the first. It is obvious such did not obtain and, therefore, the possibility of no hybridization or of genetic inheritance of BI characteristics can probably be ruled out.

The results do not rule out the possibility of the inheritance of one or more cytoplasmic factors, such as Sonneborn (1949) termed "plasmagenes," and Ephrussi (1953) discussed in a review of nucleo-cytoplasmic relations. Such a factor present in the M strain, for example, might limit the reproduction of the parasite in the fixed tissues. If all M × BI hybrids behaved, because of this cytoplasmic factor, as M strain, the BI strain characteristic of producing late tissue forms would become rarer with each hybridization until it would disappear. The number of hybridizations required for it to disappear would depend partly on chance and partly on the relative abundance of gametocytes produced by the 2 strains. It is our experience that even under the best of conditions it is more difficult to infect mosquitoes with the BI strain than with the M strain, and that gametocytes are extremely rare (about $3/10^4$ parasites) in this strain compared with those in the M strain (almost $4/10^2$ parasites). Therefore, a combination of a cytoplasmically-inherited factor carried by the M strain and a disproportion in gametocyte production is offered as a possible explanation for our results.

Still to be explained are the following facts: (1) the BI strain does, if properly manipulated, produce normal erythrocytic parasites, including gametocytes; (2) the SP strain (Coatney *et al.*, 1945) produces both late tissue forms and erythrocytic parasites when passaged through the mosquito. It is possible that the differences among the strains may be quantitative rather than qualitative or that more than one cytoplasmic factor is involved.

SUMMARY

Two successive mosquito passages of the combined M and BI strains of *Plasmodium gallinaceum* were effected by suppressing tissue forms during the F_1 infection; this allowed for a possible second hybridization of the strains. The original donor chick had been infected with the two strains by blood inoculation, and mosquitoes were allowed to feed on it. Sporozoites from these mosquitoes were used to infect chicks which were treated 9 days after inoculation with pyrimethamine (0.016 mg./gm.). In this F_1 infection gametocytes and some tissue forms were observed, approximately one-third of the chicks exhibiting brain forms either before or shortly after treatment, or at autopsy. Mosquitoes were allowed to feed on these chicks on or after day 20, and the sporozoites were used for a second mosquito passage which resulted in erythrocytic infections only, no late tissue forms being observed when brain smears were examined after the chicks were killed.

References

Coatney, G. R., Cooper, W. C., and Trembley, H. L. 1945 Studies on *Plasmodium gallinaceum* Brumpt. III. The incidence and course of infection in young chicks following the subcutaneous inoculation of pooled sporozoites. Am. J. Hyg. **42**: 323–329.

———, Cooper, W. C., Eddy, N. B., and Greenberg, J. 1953 Survey of Antimalarial Agents. Public Health Monograph No. 9, U. S. Government Printing Office, Washington, D. C.
Ephrussi, B. 1953 Nucleo-cytoplasmic relations in micro-organisms. Oxford University Press, Amen House, London E.C. 4. 127 pp.
Greenberg, J. and Trembley, H. L. 1954 Infections produced by mixed strains of *Plasmodium gallinaceum* in chicks. J. Parasitol. **40:** 336–340.
———, ———, and Coatney, G. R. 1953 The effect of pyrimethamine on the BI strain of *Plasmodium gallinaceum.* Am. J. Trop. Med. & Hyg. **2:** 771–776.
Haas, V. H., Wilcox, A., Laird, R. L. Ewing, F. M., and Coleman, N. 1948 Symposium on exoerythrocytic forms of malarial parasites. VI. Response of exoerythrocytic forms to alterations in the life cycle of *Plasmodium gallinaceum.* J. Parasitol. **34:** 306–320.
Lewert, R. M. 1948 Alteration in the development of *Plasmodium gallinaceum* following passage through tissue culture. Science **107:** 250–252.
———1950 Alterations in the cycle of *Plasmodium gallinaceum* following passage through tissue culture. I. Tissue culture studies. Am. J. Hyg. **51:** 155–177.
Sonneborn, T. M. 1949 Beyond the gene. American Scientist **37:** 35–59.
Trembley, H. L., Greenberg, J., and Coatney, G. R. 1951 Strain differences in *Plasmodium gallinaceum.* II. Experiences with the sporozoite and single oocyst passage of the BI strain. J. Nat. Malaria Soc. **10:** 68–75.
———, ———, and ———1951a Strain differences in *Plasmodium gallinaceum.* III. The spontaneous conversion of a phanerozoite-producing SP strain to a phanerozoiteless M strain through mosquito passage. J. Nat. Malaria Soc. **10:** 76–81.

RESEARCH NOTES

EFFECT OF CARBARSONE, CHINIOFON AND HIGH PROTEIN DIET ON TRICHOMONADS IN THE INTESTINE OF THE GOLDEN HAMSTER

Studies in these laboratories show that golden hamsters are often heavily infected with trichomonads and not infrequently harbor *Giardia muris* and *Endamoeba muris.* These hamsters, used in dental caries research, are maintained almost exclusively on a high carbohydrate diet. This kind of diet has been shown by Hegner and Eskridge (1937, J. Parasit. **23:** 105–106) to create favorable conditions for existence of similar parasites in the intestine of rats. Vigorous inbreeding studies with hamsters on a high carbohydrate diet have presented the possibility that vitality of the animals may be diminished and serious consequences result from a disturbance of the balance between host and intestinal protozoa. In order to prepare for such an eventuality it was considered advisable to investigate methods found efficacious in rats to determine their value in removing these parasites from hamsters.

Gabaldon (1936, Am. J. Trop. Med., **16:** 621–639) reported that 150 mg. of carbarsone per kg. of body weight administered orally to rats each day for 5 days eliminated trichomonads. Nelson and Tatum (1938, J. Pharmacol. and Exper. Therap., **63:** 122–142) claimed that chiniofon acted as a well tolerated trichomoniacidal drug in rats. Hegner and Eskridge (1937, J. Parasit., **23:** 105–106) stated that *Endamoeba muris* is eliminated from rats fed on a high protein diet. Ratcliffe (1928, Am. J. Hyg., **8:** 910–934) concluded that the number of trichomonads in rats fed a high protein diet was inversely proportional to the abundance of proteolytic anaerobic bacteria which were stimulated by protein and (1929, Science, **70:** 286–287) reported that removing young rats at 17 days of age from the mother and keeping them free from contamination, resulted in a protozoa-free colony of rats. This latter procedure would not be practical in a colony of hamsters since trichomonads have been observed in the intestine of 6 day old hamsters in this laboratory.

Trichomonad infection in the intestine of 32 golden hamsters was established by noting these flagellates in the soft feces after feeding sunflower seeds or bread soaked in a saturated solution of magnesium sulfate. These animals were treated as follows. Each of 4 hamsters maintained on diet A (45% wheat flour, 30% whole powdered milk, 5% alfalfa, 2% dried yeast, 2% liver powder, 14% corn starch and 2% Wesson's modification of the Osborne-Mendel salt mixture (1932, Science, **75:** 339–340), minus the sodium fluoride was given 15.6 mgs. of carbarsone in 0.5% sodium bicarbonate solution per day for 21 days by introducing the drug into the stomach per os by means of a small syringe and polyethylene capillary tubing (1 mm. bore). Each of 4 others on diet A was given 72.8 mgs. of chiniofon in water per day for 21 days using the same method of administration. Four others on diet B (Dog pellets—Millers Eaties 24% protein—carrots and lettuce) each received 16.25 mgs. of carbarsone in 0.5 per cent sodium

bicarbonate solution per day in their drinking water for 21 days. Similarly, 78.0 mgs. of chiniofon was added to the drinking water of each of 4 other hamsters on diet B each day for 21 days. A high protein diet consisting of 45% ground beef liver, 42% ground beef muscle, 11% lard and 2% calcium carbonate was fed daily for 21 days to 8 other trichomonad infected hamsters. Two groups of 4 hamsters each were used as controls. During the experimental period (21 days) one group was kept on diet A and the other group on diet B.

Seven days after the experiment was started microscopic examination of feces from all animals following the feeding of sunflower seeds or bread soaked in a saturated solution of magnesium sulfate revealed 1. absence of trichomonads in hamsters fed the high protein diet, 2. diminished numbers of trichomonads in hamsters administered carbarsone, 3. large numbers of trichomonads in hamsters given chiniofon and 4. large numbers of trichomonads in all control animals. One week later laparotomies (cecum) performed on one animal from each of the groupings revealed similar findings. Autopsies of all animals at the close of the experiment confirmed the above-mentioned findings. All observations were made of fecal smears prepared by thoroughly mixing approximately one cu. mm. of feces with one drop of physiological saline (0.85% NaCl) and adding a No. 1 cover slip. Using a 15× ocular and 10× objective lens, 10 fields were examined. When 5 or more fields of a smear were observed to be heavily crowded with trichomonads the term "large numbers" was employed to denote the degree of parasitism by this flagellate. Samples showing less than 5 heavily crowded fields were designated by the term "diminished numbers." Absence of trichomonads in animals was concluded upon failure to demonstrate a single trichomonad in separate smears from feces and subsequently in smears from various regions of the intestine at autopsy. A gain in weight was recorded for most animals for the duration of the experiment. Two of the 4 control hamsters on diet A and 3 of the 8 hamsters that received chiniofon suffered a slight loss in weight. *Giardia muris* and *Endamoeba muris* observed at the beginning of the experiment in each of the 8 hamsters placed on the high protein diet were not demonstrable in cecal contents obtained by laparotomy or by examination of all intestinal contents at autopsy.

This work was supported by grants from the University of Rochester and the Medical Research and Development Board, Office of the Surgeon-General, Department of the Army, under Contract No. DA-49-007-MD-495.—Wayne W. Wantland, *Department of Biology, Illinois Wesleyan University, Bloomington, Illinois, and* Erling Johansen, *Division of Dental Research and Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.*

MULTIPLE REPRODUCTIVE ORGANS IN *ASCARIS LUMBRICOIDES*

Recently Monaco and Mizelle (1953, J. Parasitology, **39**: 222) published a brief note on the occurrence of duplicated uteri in *Ascaris lumbricoides* var. *suum*.

A single similar case has now been observed during an analytical and metabolical study of the ovaries of *A. lumbricoides* var. *suum* in which over 500 worms were dissected. In this instance three complete branches of the reproductive organs (ovary, oviduct and uterus) were observed. Two of the uterine branches ramified in the usual manner, while the third branch was joined close to the base of one of these uterine branches.

Monaco and Mizelle gave no details of the branching in their note, duplication of uteri alone being discussed. Accordingly, it is impossible to make a comparison of their observations with mine. However, the present instance of multiple reproductive organs, together with the 3 other cases where multiple uteri were reported (see Monaco and Mizelle, 1953), indicate that multiple reproductive organs occur in *A. lumbricoides* from different localities as a variant from the normal type with two branches of the reproductive organs.—John K. Pollak, *Institute of Parasitology, McGill University,* (17) *Macdonald College, Province of Quebec, Canada.*

THE LIFE CYCLE OF *NEMATOSPIROIDES DUBIUS* BAYLIS (NEMATODA: HELIGMOSOMIDAE).

Previously only two life cycles have been described for heligmosomids; by Yokogawa (1922, Parasitology **14**: 127–166) for *Nippostrongylus muris,* and Schwartz and Alicata (1935, J. Wash. Acad. Sc. **25**: 128–146) for *Longistriata musculi.* The life cycle for *Nematospiroides dubius* has been determined and is here described. *N. dubius* was collected by Spurlock (1943, J. Parasitol. **29**: 303–311) from feral *Mus musculus,* and by the writer from *Peromyscus maniculatus gambelii* near Davis, California in 1950. The latter constitutes a new host record.

The eggs, measuring 70–84 microns long by 37–53 microns wide, are passed in the feces of the host in the 8–16 cell stage, and become fully embryonated 8–10 hours before hatching. At temperatures of 23–28° C. hatching occurs approximately 26 hours after passing from the host, and only in fecal material which has been kept moist.

The first stage larvae measure 300–600 microns long. Three rudimentary lips are visible around the buccal orifice. The esophagus is oxyuroid in form. The first ecdysis starts 48 hours after hatching and at this time the first stage skin can be seen loosening from both ends of the larva. Ecdysis is not complete outside the host, the first stage skin being retained as a sheath for the infective larva.

The free-living larval stages require 4–6 days to become infective larvae. At this time the latter proceed to the edge of the culture dish and assume a vertical stance using the tail as a foot. They remain motionless for long periods. Mechanical vibration, air currents and heat stimulate activity. The infective larvae measure 480–563 microns long.

Entry into the host is by ingestion, since tests indicate that larvae are unable to penetrate the skin. Upon ingestion the larvae lose their sheaths, and 24 hours later are found in the small intestine closely associated with the mucosa. At this time they measure 376–540 microns long.

From 24–48 hours following ingestion the larvae penetrate the intestinal mucosa and come to lie close to the longitudinal muscle layer of the gut wall. The worm foci are grossly visible as seen from the outside of the intestine. The first evidence of an approaching moult is seen 48 hours after infection. Ninety-six hours after infection most larvae are in the 2nd parasitic stage as pre-adults.

Rapid growth occurs and a third moult is accomplished during the latter pre-adult stages during the 6–8 days after which the worms regain the intestinal lumen and develop to egg laying maturity. Adults are tightly coiled in a spiral, males measuring 8–10 mm. and females 18–21 mm.

Eggs first appear in the feces 9 days after infection. The cycle from egg to egg takes about 15 days. Eggs are produced up to 8 months after infection. No eggs were produced 9 months after infection in one strain of mice. Variations in the lengths of the pre-patent and the patent periods are induced by host strain and also age of the host.—Frank A. Ehrenford, *Pitman-Moore Co., Indianapolis, Indiana.*

A NEW EFFECTIVE ANTHELMINTIC FOR CANINE *TRICHURIS*

In testing a series of compounds for anthelmintic action, 3-methyl-1-pentyn-3-yl sodium phthalate, (P-403), showed significant anthelmintic activity against canine *Trichuris.* The activity of this compound is specific for *Trichuris* in oral dose.

Anthelmintic activity was judged on the basis of total activity. By this criterion, Dose-Effect data for the compound gave the following results based on ova in fecal flotation and autopsy examination:

Dose Mg/Kg	No. dogs	No. positive post dose fecals		No. worms at autopsy	Percent dogs cleared
		#1	#2		
100	5	4/5	3/4*	0, 1, 15, 36, 50	20
125	6	2/5	0/3**	0, 0, 0, 0, 1, 1	67
150	10	2/10	1/10	0, 0, 0, 0, 0, 0, 0, 0, 5, 23	80
200	9	3/9	1/9	0, 0, 0, 0, 0, 0, 0, 0, 35	89

* 1 fecal not examined
** 3 fecals not examined

Lethal-Dose$_{50}$ trials, with doses of 750, 1000, 1500, and 3000 mg/kg, indicated that emesis functions as a protective mechanism. Thus, an LD_{50} was not obtained by oral dose. Very few anthelmintics possess this type of activity

These data indicate a substance, unrelated to known anthelmintics, with interesting anthelmintic properties.—Frank A. Ehrenford, *Pitman-Moore Company, New Augusta, Indiana.*

SOME HELMINTHS PARASITIC IN *CLEMMYS MARMORATA*

During the period of May to October, 1953, 64 Pacific terrapins, *Clemmys marmorata* Baird and Girard, were dissected for helminth parasites. Fifty-two of the turtles had been trapped on the Rogue River drainage in Jackson County, Oregon, and 12 came from the Calapooya River in Linn County, which is a part of the Willamette River drainage, also in Oregon.

A total of 1,236 trematodes and nematodes were collected, comprising 7 species, of which 6 apparently represent new host records.

Two species of monogenetic trematode were encountered. About 32% of the turtles contained *Polystomoides coronatus* Leidy, and 3% were infected with *Neopolystoma orbiculare*

Stunkard. Among the digenetic flukes found was *Spirorchis artericola* Ward which was present in 14% of the turtles examined.

In addition, 12 specimens of a Paramphistomid fluke, tentatively identified as *Ophioxenos dienteros* Sumwalt, were taken from the cloacal area of 3 Calapooya River terrapins. This fluke was described from a garter snake, *Thamnophis* sp., and present material represents the first record for the species in a chelonian host.

The commonest digenetic fluke found in these turtles was the intestinal fluke *Telorchis corti* Stunkard. This species had been previously reported by Ingles (J. Parasit. **17**: 101–103; 1930) from a Pacific terrapin in California. Present specimens showed an interesting age, or size, immunity to infection by this species. A greater incidence of infection, as well as larger infections, was encountered in the smaller turtles. Of the 16 terrapins from 10–13 cm. in carapace length 81% were parasitized with *T. corti* while the 31 specimens between 13 and 16 cm. showed only 10% of infection by this species. Four terrapins larger than 16 cm. were examined and none contained any specimens of this fluke.

Two species of nematode were encountered in *C. marmorata*. Some 31% of the turtles contained *Spironoura affine* Leidy, and 84% harbored *Spiroxys contortus* Rudolphi. Neither of these nematodes has previously been recorded from this host.—Vernon E. Thatcher, *Zoology Department, Oregon State College, Corvallis, Oregon.*

THE USE OF A SURFACE ACTIVE AGENT TO FACILITATE THE EXAMINATION OF INTESTINAL CONTENTS FOR HELMINTH PARASITES

During the course of quantitative studies on *Nippostrongylus muris* (Nematoda: Heligmosomidae) infections in laboratory rats, the accurate determination of worm burdens was hindered by the difficulty encountered in separating the parasites from mucous and other intestinal material. The usual procedure consisted of first scraping the mucosa and gut contents from a two to three inch segment of intestine into a counting plate. A small amount of water was added and the material was finely broken up with a bent glass rod and examined under a dissecting microscope.

The substitution of a nonionic surface active agent, Tween 80, for water greatly facilitated the procedure. The addition of 4 to 5 cc. of a 25 percent aqueous solution of the Tween 80 stock not only reduced the time required for breaking up the mucous and other intestinal material but also yielded a finer more easily examined preparation. The net result was that longer segments of intestine could be processed with greater accuracy in less time. The worms were not visibly injured by the treatment and remained viable throughout the period of counting and study.

There has been no opportunity to evaluate the method with other intestinal helminth infections and no comparative study with other surface active agents has been made. The present technic or modifications of it, however, should prove helpful in situations where a similar type of experimental design is employed.—A. James Haley, *Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.*

FURTHER OBSERVATIONS ON *GLUGEA HERTWIGI* WEISSENBERG 1911, 1913 (MICROSPORIDIA) IN FRESH WATER SMELT IN NEW HAMPSHIRE

Because of its high value as a forage and food fish the restricted natural distribution of the fresh water smelt, *Osmerus mordax* (Mitchell), in New Hampshire has been greatly extended by transplantation. Each year during the spring spawning runs large quantities of smelt and smelt eggs are collected by the N.H. Fish and Game Department from Lake Winnisquam and distributed to other bodies of water throughout the state. During the late winter and spring of 1952, 910 smelt from Winnisquam were examined of which 86 (9.5 percent) were found infected with *Glugea hertwigi*. These data are particularly interesting when viewed according to the time of collection. Thus: February 9 (230 examined, none infected); April 22 (269 examined, 15 [5.6 percent] infected); May 9 to 19 (411 examined, 71 [17 percent] infected. Although the data appear to suggest a seasonal factor in the host-parasite relationship further study is needed to clarify this point.

O. mordax in N.H. is believed to be indigenous only in 2 or 3 bodies of water, one of which is Winnisquam, but through transplantation operations it has been introduced into nearly 100 other lakes and ponds. Since *G. hertwigi* was demonstrated in the transplanting stock at Winnisquam it was not surprising to find infected smelt populations elsewhere. Examination of nearly 500 smelt from several other localities, whose smelt populations were established from Winnisquam stock, showed the disease to be present in 7 additional lakes and ponds. Thus, to date the infection has been demonstrated in about 10 percent of the smelt populations in the state. The majority of localities, however, have not been studied and future surveys undoubtably will show diseased smelt in many of them.

Previous work by the author (Haley, J. Parasit. **38**: 183; 1952 and Trans Amer. Fish. Soc. In Press) and other investigators has demonstrated the severe pathological manifestation of the infection and in view of its extensive distribution and continued dissemination it is a problem of sufficient consequence to warrant serious consideration by individuals concerned with fisheries research in New Hampshire.—A. James Haley, *Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.*

A NEW RECORD OF THE CESTODE *INFULA BURHINI* BURT (CYCLOPHYLLIDEA: DIOECOCESTIDAE) FROM AUSTRALIA

Among specimens kindly lent to us by Dr. E. W. Price, we found at least two fragmented cestodes (U. S. Nat. Mus. Helm. Coll. No. 33415) which are without doubt *Infula burhini* Burt, 1939 (Spolia Zeylanica, **21**: 195–208. The cestodes were collected by Dr. N. A. Cobb from the small intestine of a White-headed Stilt (*Himantopus himantopus leucocephalus*) in Australia. Precise locality data are not available. To our knowledge, *Infula burhini* has not been reported since its original description by Burt from *Burhinus oedicnemus* and *Himantopus himantopus* in Ceylon. Our observations on one male and one female specimen, agree in all particulars with those recorded by Burt (1939).—Marietta Voge, and Clark P. Read, *Departments of Infectious Diseases and Zoology, University of California, Los Angeles.*

SOME RECORDS OF ECTOPARASITIC ACARINA AND INSECTA FROM MAMMALS IN ONTARIO

In two communications previously published (Jour. Mammalogy, **31**: 357–358, 1950; **34**: 137–139, 1953) the writer reported upon collections of ectoparasitic ticks, mites and insects taken from mammals in southern Ontario. Since then a variety of specimens taken by several collectors from mammals in Ontario have been added to the collections of the University of Western Ontario. Most of the specimens have been mounted in Canada balsam on microscope slides, and in the following account the catalogue numbers of the slides are included. The name of the collector, where known, is also recorded. Each dated entry represents a collection from a single host. Identifications of the specimens were kindly made by the following taxonomists: G. F. Ferris, Natural History Museum, Stanford University, California (Anoplura), J. D. Gregson, Laboratory of Entomology, Department of Agriculture, Kamloops, B. C., (Ixodidae), G. P. Holland, Systematic Entomology, Department of Agriculture, Ottawa (Siphonaptera), H. H. J. Nesbitt, Department of Biology, Carleton College, Ottawa (Laelaptidae) and R. A. Ward, Department of Zoology, University of Chicago, Chicago, Illinois (Mallophaga).

ACARINA

Laelaptidae

Laelaps muris Ljungh—several mites from *Microtus p. pennsylvanicus* (Ord), Komoa, January 2, 1933, E. Davis (slide E16.49); one mite from *M. p. pennsylvanicus* (Ord), London Twp., Middlesex Co., February 4, 1952 (E16.62).

Laelaps microti (Ew.)—several mites from *Microtus p. pennsylvanicus* (Ord), Komoka, January 2, 1933, E. Davis (E16.49); several mites from *M. p. pennsylvanicus* (Ord), Melrose, December 21, 1940, N. R. Brown (E16.55).

Ixodidae

Ixodes cookei Packard—1 ♀ from *Mephitis mephitis* (Schreber), Byron, November 13, 1932, E. Davis (E16.48); 1 engorged ♀ from *Felis domesticus* L., a young kitten to which the tick was firmly attached in a pit in the skin between the right shoulder and the back, Delaware Twp., Middlesex Co., July 22, 1952, D. A. McLarty (E16.15); 1 ♀ from *Castor canadensis* Kuhl., Arden Lake, Arden Twp., Sudbury Dist., May 11, 1953, W. Punstel; 2 ♀♀ from *Marmota monax* (L.), a young animal of the year, London, June 18, 1953, W. W. Judd (E18.20, 21).

ANOPLURA

Haematopinidae

Hoplopleura acanthopus (Burm.)—8 lice from *Microtus p. pennsylvanicus* (Ord), London Twp., Middlesex Co., February 4, 1952 (E16.60, 61). G. F. Ferris (The sucking lice. Mem. Pacific Coast Entomol. Soc., vol. 1, 1951) reports this species from several spp. of *Microtus*, not including *M. pennsylvanicus*.

Neohaematopinus sciurinus (Mjöberg)—3 lice from *Sciurus carolinensis leucotis* (Gapper), London, December 6, 1933, E. Davis (E16.36).

MALLOPHAGA

TRICHODECTIDAE

Eutrichophilus setosus Giebel—numerous ♂♂ and ♀♀ from *Erethizon dorsatum* (L.), Atikokan, June 2, 1933, E. Davis (E16.56–59). This sp. was recorded from *E. dorsatum* in Nova Scotia by G. B. Thompson (Can. Entomol., **66**: 279–281, 1934).

Trichodectes canis (DeGeer)—numerous ♂♂ and ♀♀ from *Canis* (*lupus* L.?), Maple, January 29, 1953 (E16.63–65). The host was a pup, taken from a den near Elizabethtown on February 26, 1952, and may have been an offspring from a mating of a wild wolf and a domestic dog. The parasites were removed from it on its death at Maple on January 29, 1953 after it had been kept in captivity since its original capture. G. B. Thompson (Can. Entomol., **66**: 279–281. 1934) records *T. canis* from a timber wolf at Hearst, Ont.

SIPHONAPTERA

HYSTRICHOPSYLLIDAE

Nearctopsylla g. genalis (Baker)—3 ♂♂, 1 ♀ from *Mustela erminea cicognanii* Bonaparte, December 28, 1932, Hough Lake, E. Davis (E16.40, 41).

Tamiophila grandis (Rothsch.)—1 ♀ from *Tamias striatus lysteri* (Richardson), September 2, 1932, Penetanguishene, E. Davis (E16.42).

CERATOPHYLLIDAE

Orchopeas caedens (subsp. *durus* ?) (Jordan)—1 ♀ from *Mustela vison* Schreber, March 20, 1953, Chaplin Twp., Sudbury Dist., T. Cachagee (E18.26). G. P. Holland (Siphonaptera of Canada. Dept. Agric., Tech. Bull. 80, 1949) lists this flea as a parasite of squirrels and includes some cases of its occurrence on *Mustela* spp. In the latter case the parasites presumably move to the predator from squirrels being devoured.

Orchopeas leucopus (Baker)—numerous ♂♂ and ♀♀ from *Peromyscus* sp. (captives in laboratory cages), May 19, 1951, London, R. H. Stinson (E17.92–100, E18.1–5).

COLEOPTERA

LEPTINIDAE

Leptinillus validus (Horn)—8 larvae from *Castor canadensis* Kuhl., January 21, 1954, Twp. 35, Chapleau Forestry Dist., V. Crichton (E18.22–25). The specimens were identified with keys in H. F. Chu (How to know the immature insects. W. C. Brown Co., Dubuque, Iowa, 1949). G. H. Horn described this species as *Leptinus validus* (Trans. Amer. Ent. Soc., **4**: 145–146, 1872) and discussed it further (Trans. Amer. Ent. Soc., **10**: 113–114, 1882), his specimens being from northern Ontario.—W. W. JUDD, *Dept. of Zoology, University of Western Ontario, London, Ontario.*

NOTES ON THE RABBIT TICK, *HAEMAPHYSALIS LEPORIS-PALUSTRIS* (PACKARD), AND TULAREMIA IN CENTRAL ALASKA

The first published reports on the distribution of the rabbit tick in Alaska resulted from investigations conducted during June and July of 1937. At that time it was found that "infestations of the rabbit tick, *Haemaphysalis leporis-palustris,* were remarkably localized." Although hares were examined at localities scattered all along the Steese and Richardson Highways and the Alaska Railroad, ticks were found only on hares from Seward and the immediate vicinity of College, northwest of Fairbanks (Philip, C. B.: J. Parasit. **24**: 483–488, 1938). *Bacterium tularense* was recovered from 3 samples of ticks from the College area (Philip, C. B. and R. R. Parker: Pub. Health Rep. **53**: 574–575, 1938).

No further studies were conducted until 1950, when a project was initiated by the Arctic Aeromedical Laboratory, Ladd Air Force Base, to investigate potential disease vectors in Alaska. During 1950 to 1952, *H. leporis-palustris* were collected from a total of 47 hares, but no *B. tularense* was found in these collections when tested by guinea pig injection. The ticks were distributed over a wider range than in 1937, at least as far as Circle Hot Springs to the northeast, Big Delta to the southeast, and the village of Clear to the southwest of Fairbanks.

The 1953 tick collections extended these earlier distribution records only to the west and northwest. Two ticks were removed from a hare found dead at Minto, and all of 19 hares examined at Livengood were infested. Despite the previous record from Circle Hot Springs, the ticks have apparently not become well established in the Central-Circle area, for all of the 29 hares examined from this area during 1953 were free from ticks.

Fourteen lots with a total of 430 *H. leporis-palustris* collected during 1953 were tested at

the Rocky Mountain Laboratory by injection of suspensions into guinea pigs. Aliquot portions of about half of these suspensions also were injected into baby mice to check for the presence of Colorado tick fever virus, but results of the latter tests were all negative.

Three of the above 14 tick lots yielded isolates of *B. tularense* in test guinea pigs (pure cultures were obtained after passage of each). Characteristic gross lesions also were observed at necropsy of these animals. The data on the ticks used in each of these 3 tests were as follows:

1. Lot 38: 24 adults and 3 nymphs taken from a pool of 271 ticks removed from 2 apparently healthy hares at College on May 19.
2. Lot 39: 5 females, 4 males, and 5 nymphs taken from a hare found dead in the College area by a University of Alaska student on May 21.
3. Lot 54: 1 engorged female taken from a sick hare found in the College area by a University of Alaska student on June 25.

Facilities for testing tissues from these two affected hare hosts unfortunately were not available at the time of discovery.

The presence of the low-grade type of Rocky Mountain spotted fever found in *H. leporis-palustris* studied previously at this Laboratory from other Nearctic areas, was suspected during the 1937 investigations in Alaska (Philip, C. B., 1938, l.c.). Similar findings also occurred in the tick tests during 1953. Two lots of ticks, which were treated with antibiotics to suppress contaminants, were injected directly into and passed in embryonated chicken eggs. Rickettsiae were observed in Giemsa-stained smears of the yolk sacs. Data on the tick lots were as follows:

1. Lot 37: 170 ticks (all stages) taken from 16 hares at Livengood on May 14.
2. Lot 38: 219 ticks (all stages) taken from the pool of 271 removed from two hares at College (different from the 27 ticks of this lot used for the tularemia tests reported above).

The egg-propagated rickettsial isolates, when injected into guinea pigs, were avirulent, caused no scrotal involvement, and could not be maintained in serial passage. However, 7 guinea pigs inoculated with these avirulent strains showed complete immunity on challenge with a fully virulent laboratory strain of Rock Mountain spotted fever, and the original infectious exposure of these animals followed by recovery was confirmed by positive complement fixation in sera from three animals before challenge.

From available data, it is evident that *H. leporis-palustris* has extended its known range in central Alaska, and it is possible that tularemia may show up in other areas through this medium. However, the very limited amount of research done on this problem in the Territory allows one to suspect that tularemia may already be present in other areas but simply has not been discovered. Rabbit ticks can be expected to fluctuate in abundance and distribution in accordance with well known hare cycles, and availability of hosts in a given range at different periods in such cycles. Two cases recorded by MacKinnon (J. Can. Med. Assoc. **56**: 541–542, 1947) from the Norman Wells region of the Mackenzie River Valley of Canada may indicate a wider distribution of tularemia in northern latitudes than is presently known.

Although a possible but unconfirmed case of tularemia from the vicinity of Wiseman was diagnosed in 1938 (Philip, C. B.: Proc. Sixth Pacific Sci. Cong. **5**: 71–73, 1939), the first serologically confirmed case of tularemia in Alaska following the handling of muskrats near Northway, was reported by Williams (Pub. Health Rep. **61**: 875–876, 1946). To date, 8 cases of tularemia from the Fairbanks-Steese Highway area have been diagnosed and confirmed (Pauls, F. P.: Significant Diseases in Alaska, Data Compiled for Indoctrination of Medical Officers at Elmendorf Air Force Base, Alaska, 1953). Incidental to our studies in 1951, serums from 3 old-time residents were tested for *B. tularense* agglutinins with negative results. These men had handled many hares while trapping and acting as big game guides between 20 and 30 years in several areas in Alaska.

Although this "increase" in human cases in recent years could be due to a greater awareness among local physicians of the presence of tularemia in this area, it is possible that the problem of tularemia is becoming more serious with increasing settlement and widening exposure. Since there are no ticks in Central Alaska that attack man, the chief source of human infection will continue to be the handling of snowshoe hares or other infected animals. Biting flies could be of incidental importance in the local presence of a hare epizootic, though tularemia is still not a known primary cause for widespread decimation of hares and rabbits (Philip, C. B., Bell, J. F., and Larson, C. L., 1953, XIV Intern. Zool. Cong. Copenhagen, in press). The problem affords an excellent opportunity for further research and it is expected to receive continuing attention by research personnel in Alaska.—Cornelius B. Philip, *Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana;* Gordon D. Gill, 1st Lt., USAF (MSC), *Arctic Aeromedical Laboratory, Ladd Air Force Base, Alaska,* and John M. Geary, Maj., USAF (MSC), *now at Cornell University.*

EFFECTS OF DIETARY PROTEIN ON THE RELATIONSHIP BETWEEN LABORATORY MICE AND THE NEMATODE *NEMATOSPIROIDES DUBIUS*

Quantitative studies were conducted during 1950–52 on the effects of 10 and 20% dietary protein levels on the relationship between heterozygous albino female mice and *Nematospiroides dubius* (NEMATODA: HELIGMOSOMIDAE). These investigations were carried out under conditions of initial challenge of the host by *N. dubius*.

N. dubius used in these tests was recovered from *Peromyscus maniculatus gambelii* near Davis, Yolo County, California in 1950.

N. dubius was selected as the parasite because of several desirable characteristics: it has been shown to be pathogenic to mice by Spurlock (1943, J. Parasit. **29**: 303–311); infection occurs by ingestion; it produces a rapid host reaction; it is limited to gut wall tissue invasion; it persists in the host for about 8 months; and it is cultured with ease as a pure population in large numbers.

Mice in lots of 10 were tested for the effects of *N. dubius* on the growth curve as compared to uninfected controls: 100 larvae produced a slight but measurable temporary depression; 200 larvae produced a greater depression which was followed by an accelerated phase; 400 larvae caused 1 death in 20 days; 800 larvae caused 5 deaths in 20 days. A standard dose of 100 larvae per mouse was used in the subsequent tests.

It was found that 10 and 20% protein diets produced similar growth curves in mice, and that these curves were comparable to growth curves of mice on a commercial diet. No evidence was found by histopathological examination of protein depletion in mice on these two levels of dietary protein in 20 days. A diet containing less than 10% protein was found to be insufficient for maintenance.

Infected mice on the 20% protein level were protected against the effects of *N. dubius*, based upon growth curves and blood cellular changes. Infected mice on the 10% protein level suffered a temporary weight loss, and more severe blood changes than mice on the 20% protein diet. The effects of *N. dubius* on growth and the blood cellular elements were found to occur after the pre-patent period and at the onset of ovum production.

Diet appeared to have an appreciable effect on the survival and location of *N. dubius*. In older mice, 8.5% less worms survived on the 10% protein level than on the 20% protein; subsequent examination showed that these limited observations were not statistically significant. However, the 10% protein level restricted the distribution of the adult worms to a more anterior location than the 20% protein level.

An appreciable splenomegaly occurs in infected mice. Examination of liver and spleen sections revealed no discernible pathology attributable to *N. dubius*.

No evidence was found of secondary bacterial invasion in the gut lesions produced by *N. dubius*.—FRANK A. EHRENFORD, *Pitman-Moore Company, New Augusta, Indiana.*

News Note

THE SIXTH ANNUAL CONFERENCE OF MIDWESTERN PARASITOLOGISTS

The conference was held at Michigan State College June 21st and 22nd, 1954. There were 83 registered and people attending came from as far west as Kansas and south from Missouri and east from the Atlantic seaboard. A program of demonstrations totaling 18 varied presentations employed the first afternoon of the meeting. The banquet was held at the Kellogg Center where Dr. G. F. Otto was the speaker of the evening on the topic: "Mosquitoes, Worms, Samoans and the Parasitologist in Samoa." The address was punctuated with a fine series of Kodachrome slides. The second day of the meeting consisted of a business meeting in which Dr. R. M. Cable was elected presiding officer for the coming year. After the election everyone enjoyed a graduate student panel discussion of problems in the training of advanced students in parasitology.

The committees on next year's meeting place and program were not able to come to a decision so this will be announced later in the year.

This organization, although primarily in the midwest, welcomes any interested persons to attend and participate in the meetings.—William D. Lindquist, Sec.-Treas.

Dept. of Bacteriology
Michigan State College
East Lansing, Michigan.

Contents—(continued)

Research Notes:

Effect of Carbarsone, Chiniofon and High Protein Diet on Trichomonads in the Intestine of the Golden Hamster: **Wayne W. Wantland and Erling Johansen.** Multiple Reproductive Organs in *Ascaris lumbricoides:* **John K. Pollak.** The Life Cycle of *Nematospiroides dubius* Baylis (Nematoda: Heligmosomidae): **Frank A. Ehrenford.** A New Effective Anthelmintic for Canine *Trichuris:* **Frank A. Ehrenford.** Some Helminths Parasitic in *Clemmys marmorata:* **Vernon E. Thatcher.** The Use of a Surface Active Agent to Facilitate the Examination of Intestinal Contents for Helminth Parasites: **A. James Haley.** Further Observations on *Glugea hertwigi* Weissenberg 1911, 1913 (Microsporidia) in Fresh Water Smelt in New Hampshire: **A. James Haley.** A New Record of the Cestode *Infula burhini* Burt (Cyclophyllidea: Dioecocestidae) from Australia: **Marietta Voge and Clark P. Read.** Some Records of Ectoparasitic Acarina and Insecta from Mammals in Ontario: **W. W. Judd.** Notes on the Rabbit Tick, *Haemaphysalis leporis-palustris* (Packard), and Tularemia in Central Alaska: **Cornelius B. Philip, Gordon D. Gill, and John M. Geary.** Effects of Dietary Protein on the Relationship between Laboratory Mice and the Nematode *Nematospiroides dubius:* **Frank A. Ehrenford.** (479–486)

BIOLOGICAL ABSTRACTS

Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in five low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section C—Abstracts of Microbiology, Immunology, and Parasitology—affords a very complete coverage of the Parasitologists' literature. The price is only $6.00 a year (Foreign, $6.50) including the comprehensive index issue of the complete edition.

Write for a sample copy.

BIOLOGICAL ABSTRACTS

University of Pennsylvania
Philadelphia 4, Pa.

WANTED

Your spare copies of back numbers of the

JOURNAL OF PARASITOLOGY

Recent inroads on certain issues of the Journal have depleted the copies in stock. Duplicate or unwanted issues may be returned to the Custodian of Back Issues where they will become available to new subscribers or to members who wish to complete their files. Until further notice the Society will be glad to pay transportation charges on ANY back issues. In addition, the following numbers will be purchased at the current selling price:

Vol. 18 (Nos. 1, 4)
21 (Nos. 1, 2, 3, 4)
Vol. 26 (Nos. 1, 3, 4, 5)
29 (Nos. 4, 6)

W. S. BAILEY—Custodian,
School of Veterinary Medicine,
Alabama Polytechnic Institute,
Auburn, Alabama